Modifying Factors in the Phenotypic Expression of Hypertrophic Cardiomyopathy

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Declaration

I can confirm that the writing of this thesis is entirely my own work and that the work to which it relates was my own. The final chapter on disease modification was undertaken as part of a group but I was significantly involved in all aspects of recruitment, investigation, analysis and the writing of the final manuscript. Cardiac MRI scans were performed by the Laboratory of Cardiac Energetics on site at the Clinical Centre, National Heart, Lung, and Blood Institute, National Institutes of Health. Analysis and calculation of left ventricular indices from the resultant images was undertaken by me. Genetic polymorphisms were determined in our own laboratory by me. In addition all HCM genotyping was undertaken in our own laboratory. The laboratories responsible for biochemical tests performed outside the Clinical Centre of the National Institutes of Health are named in the relevant chapter. All other biochemistry was performed on site.

Presentations and Publications

Publications

1. **Begley D**, Mohiddin S, Tripodi D, Winkler J, Fananapazir L. Efficacy of implantable cardioverter defibrillator therapy in primary and secondary prevention of sudden cardiac death in hypertrophic cardiomyopathy. *Pacing Clin Electrophysiol* 2003;26(9):1887-1896
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Presentations

1. **Begley D,** Winkler J, Plexico G, Tripodi D, Arai A, Mohiddin S, Fananapazir L. Brain natriuretic peptide in hypertrophic cardiomyopathy caused by sarcomeric gene mutations: A marker for poor outcome? *J Am Coll Cardiol* 2000;35(2):191A
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4. **Begley D**, Mohiddin S, Winkler J, Fananapazir L. The Angiotensin-I Converting Enzyme Insertion (I)/Deletion (D) Polymorphism Influences Ventricular Refractoriness in Patients with Hypertrophic Cardiomyopathy. *Pacing Clin Electrophysiol* 2001;24(4):
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Abstracts

1. **Begley D,** Plexico G, Arai A, Mohiddin S, Fananapazir L. Gender-specific cardiac phenotypic differences detected by magnetic resonance imaging in hypertrophic cardiomyopathy caused by sarcomeric gene mutations. *J Am Coll Cardiol* 2000;35(2):190A
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Contents

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Abbreviations and Acronyms

ACE	Angiotensin converting enzyme
AF	Atrial fibrillation
AG	Angiotensin
ARB	Angiotensin receptor blocker
AT_1/AT_2	Angiotensin receptors types 1 & 2
AV	Atrio-ventricular
BP	Blood pressure
BSA	Body surface area
ECG	Electrocardiogram
EP	Electrophysiologic
ET-1	Endothelin-1
HCM	Hypertrophic cardiomyopathy
ICD	Implantable cardioverter defibrillator
IGFBP3	IGF binding protein 3
IGF-1	Insulin-like growth factor -I
LV	Left ventricular
MRI	Magnetic resonance imaging
MVO_2	Maximal ventilatory oxygen consumption
NYHA	New York Heart Association
RAS	Renin-angiotensin system
SAM	Systolic anterior motion
SEM	Standard error of the mean
SCD	Sudden cardiac death
SVT	Supraventricular tachycardia
VT	Ventricular tachycardia

Abstract

Objectives

The objectives of these studies were to assess the role of potential modifying factors in determining the degree of LV hypertrophy in patients with HCM and to identify targets for intervention and to assess the ability of ACE inhibition and/or AG-II receptor blockade to cause regression of LV hypertrophy in patients with HCM.

Methods

Patients referred to the National Heart, Lung and Blood Institute, Bethesda, Maryland, for evaluation of HCM, were screened using standard echocardiographic techniques. Family members of patients with familial HCM were also screened by echocardiography and for the presence of the disease causing mutation. This allowed inclusion of patients in studies who were known to have disease causing mutations but who had no evidence of LV hypertrophy. All patients subsequently underwent cardiac MRI studies to evaluate LV mass and maximal wall thickness. LV indices were then compared with potential modifying factors to assess their role in determining phenotypic expression. Disease regression was also examined using enalapril and losartan in a randomized double-blind placebo controlled study.

Results

The gender differences in the pattern of LV hypertrophy observed in patients with HCM is similar to other forms of pressure overload hypertrophy. Women have smaller volumes and a relatively thicker myocardium compared to men. This pattern should reduce wall stress and may slow the progression to heart failure. In addition, this highlights a number of limitations including the difficulty in establishing the diagnosis in borderline cases when a single unified definition of HCM is applied to both genders.

The ability of insulin-like growth factor-1 and its binding protein (IGFBP3) to modulate cardiac hypertrophy in familial hypertrophic cardiomyopathy was examined in 150 subjects with determined genetic status. Plasma IGF-1 concentrations were lower in HCM patients. A significant inverse correlation existed between IGF-1 concentrations and maximum LV wall thickness assessed by MRI. No association was found between IGFBP3 concentrations and cardiac hypertrophy. As IGF-1 improves cardiomyocyte function, we postulate that increased IGF-1 activity causes a milder cardiac phenotype because it counteracts the myocyte contractile dysfunction induced by sarcomeric mutation, thereby attenuating the compensatory myocardial hypertrophic response.

BNP has recently emerged as a screening tool for patients with heart failure. Although it has poor positive predictive value it is a good negative predictor (97%) and therefore can be used to exclude significant heart disease in patients presenting with breathlessness. We hypothesized that levels of BNP would correlate with the severity of LV hypertrophy in HCM. This proved incorrect with no correlation observed. However, sarcomeric mutations that are likely to have a worse prognosis

(higher incidence of SCD and more frequent progression to heart failure) were associated with significantly higher BNP levels compared to mutations that followed a more benign course, independent of LV systolic function or current symptoms.

Genetic factors have been proposed as modifiers of LV hypertrophy in several disease states including HCM. We reassessed the role of two genetic polymorphisms that have previously been reported to influence the extent of LV hypertrophy in HCM. The ACE gene polymorphism consisting of the presence or absence of a long intronic section of DNA is associated with measured serum levels of ACE. HCM patients who are homozygous for the deletion have a greater degree of LV hypertrophy. However we could find no correlation between the $198^{Lys\text{-}Asn}$ ET-1 missense mutation and LV hypertrophy in HCM that has also been reported.

We hypothesized that ACE inhibition and/or AG-II receptor blockade would result in regression of LV hypertrophy in HCM. Non-obstructed HCM patients were randomized to receive enalapril alone, losartan alone, a combination of both drugs, or placebo for a period of six months. LV mass was determined before and after the study period. Enalapril, alone or in combination with losartan, but not losartan alone significantly reduced LV mass compared to placebo.

Conclusions

Identification of modifying factors in the phenotypic expression of HCM helps in our understanding of the mechanisms which explain the marked variation observed. This may provide avenues for therapeutic intervention either in the regression or prevention of development of LV hypertrophy in HCM with potentially subsequent improvement in symptoms and prognosis.

Short Abstract

The objectives of this study were to assess the role of potential modifying factors in the phenotypic expression of hypertrophic cardiomyopathy (HCM) and to assess therapeutic options that might cause regression of left ventricular (LV) mass in HCM.

A total of 269 patients were carefully phenotyped and LV mass was determined by cardiac MRI. The role of gender, insulin-like growth factor-I (IGF-I) and genetic polymorphism in the angiotensin converting enzyme (ACE) and endothelin-1 (ET-1) genes in determining LV mass in HCM was assessed. The relationship between brain natriuretic peptide (BNP) and HCM caused by known sarcomeric mutations was also assessed. The role of ACE inhibition and/or blockade was also assessed in regression of LV hypertrophy in non-obstructive HCM patients.

Female patients were found to have relatively thicker hearts with smaller cavities than male patients as in LV hypertrophy caused by pressure overload. Higher physiological levels of IGF-I were associated with lesser degrees of LVH which may be due to increased muscular efficiency. BNP levels were associated with the disease causing mutation but not to the degree of LVH. Mutations associated with an adverse prognosis had significantly higher BNP levels. The insertion/deletion (I/D) ACE gene but not ET-1 gene polymorphism was associated with LV mass in HCM. Treatment with enalapril alone or in combination with losartan resulted in a 7% and 8.5% reduction in LV mass respectively over 6 months.

HCM is a complex disease with many potential phenotypic modifiers. These studies suggest that identifying modifying factors is important to explore therapeutic

options in the management of these patients. In addition further studies exploring the long term impact of such therapies on disease progression are indicated and their role in disease prevention in pre-clinical states.

SECTION I: INTRODUCTION AND METHODS

1. Introduction

HCM is a relatively common autosomal dominant inherited cardiac condition characterized by LV hypertrophy that is inappropriate for the degree of afterload. (Maron 2002) The disease was first reported by a pathologist, Donald Teare, in 1958, when he described asymmetrical hypertrophy of the left ventricle in a number of young adults. (Teare 1958)

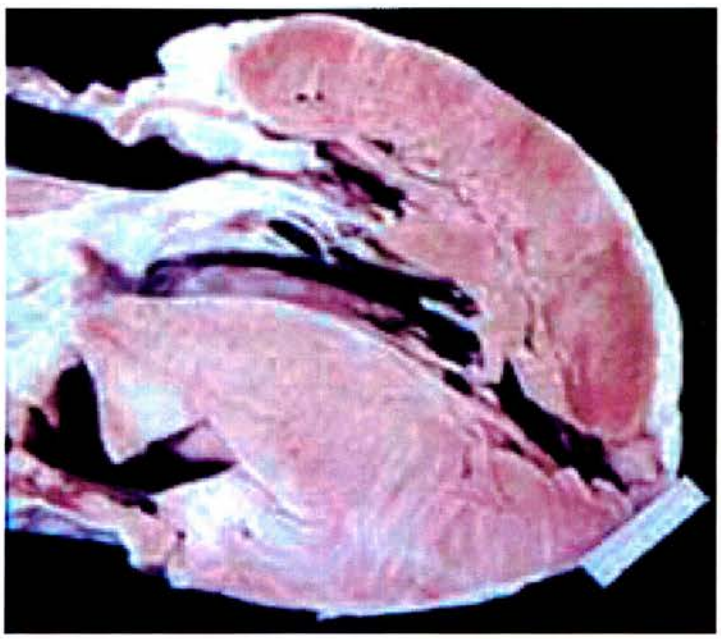

Figure 1-1: Cross section through heart of patient with HCM. Although there is marked global hypertrophy, there is disproportionate thickening of the ventricular septum.

The prevalence of echocardiographically detectable disease in an adult population is about 1:500. (Maron *et al.* 1994; Maron *et al.* 1995) Clinical diagnosis is usually established by the presence of LV hypertrophy on 2-dimensional echocardiography in the absence of other cardiac or systemic disease (e.g. hypertension or aortic stenosis) that may be capable of producing the degree of

hypertrophy identified. (Klues *et al.* 1995) In addition to the LV hypertrophy, HCM is also characterized by diastolic dysfunction, myocardial ischaemia and, supraventricular and ventricular arrhythmias. The extent of LV hypertrophy, diastolic dysfunction and myocardial ischemia is an important determinant of clinical course. (Frank and Braunwald 1968; Wigle *et al.* 1995)

Clinical course is extremely variable. The majority of subjects remain asymptomatic and therefore, presentation can be at any age. However, subjects may complain of breathlessness, chest pain, palpitations, fatigue, and symptoms of impaired consciousness (pre-syncope and syncope), resulting in impaired exercise tolerance and significant functional limitation. (Cannan *et al.* 1995; Cecchi *et al.* 1995; Fay *et al.* 1990; Frank and Braunwald 1968; Maron *et al.* 1999b; Maron *et al.* 2003a) One of the most catastrophic associations with HCM is the risk of SCD, which can occur in otherwise healthy, asymptomatic and young individuals. (Maron *et al.* 1982) Although early reports suggested mortality rates of 3-6%, (Shah *et al.* 1974) based on tertiary centre experience, more recent reports suggest a much lower rate of about 1%. (Cannan *et al.* 1995; Cecchi *et al.* 1995; Kofflard *et al.* 1993; Shapiro and Zezulka 1983; Spirito *et al.* 1989)

Approximately 5-10% of patients may progress to advanced end-stage heart failure with ventricular dilatation and wall thinning, and LV systolic dysfunction resembling dilated cardiomyopathy. (Spirito *et al.* 1987)

HCM is caused by mutations in genes that encode components of the sarcomere which has led it to be termed a disease of the sarcomere. (Thierfelder *et al.* 1994) To date, mutations in at least ten genes have been reported to cause the disease. (Bonne *et al.* 1995; Geisterfer-Lowrance *et al.* 1990; Gollob *et al.* 2001;

Hoffmann *et al.* 2001; Kimura *et al.* 1997; Olson *et al.* 2000; Poetter *et al.* 1996; Satoh *et al.* 1999; Thierfelder *et al.* 1994) An additional locus has been identified recently on chromosome 7 although the causative gene has not been determined. (Song *et al.* 2006)

Not all patients who carry a disease causing mutation will express LV hypertrophy. (Fananapazir and Epstein 1995; Marian *et al.* 2001; Maron *et al.* 1998) It is common to find no evidence of LV hypertrophy in a pre-adolescent child. However, during periods of rapid growth, development of LV hypertrophy occurs with the completed phenotype usually present by the time of physical maturity. (Maron *et al.* 1986a; Spirito and Maron 1987) However, with the advance of molecular techniques, genotype-phenotype studies have demonstrated that complete disease expression can be delayed until much later in life. This is most commonly seen in patients with mutations in troponin T or myosin-binding protein C. (Charron *et al.* 1998; Maron *et al.* 2001; Niimura *et al.* 1998; Elliott *et al.* 1999a)

In addition some patients with disease causing mutations may still have fibre disarray and diastolic dysfunction but never develop LV hypertrophy. This has been seen especially in patients with troponin T and α-tropomyosin mutations and these patients have been shown to be still at risk of SCD.

1.1. Left Ventricular Hypertrophy

LV hypertrophy is a major independent risk factor for morbidity and mortality from cardiovascular disease. (Frohlich 1991; Levy 1988) Patients with HCM have also been reported to be at increased risk for sudden cardiac death. (Maron *et al.* 1982; McKenna and Deanfield 1984) As in other disease states, cardiac

hypertrophy in HCM is due to hypertrophy of myocytes and hyperplasia of several other cell types, namely, fibroblasts, smooth muscle cells and endothelial cells. Indeed, the non-myocyte cellular hyperplasia may account for more than half of the LV mass. (Davies and McKenna 1995; Ferrans *et al.* 1972) Although cardiac myocytes are normally terminally differentiated cells, there is evidence that in adult life, myocyte hyperplasia may occur in massive LV hypertrophy. (Ferrans and Rodriguez 1987)

In the classical form of the disease as described by Teare, (Teare 1958) hypertrophy is isolated to the anterior septum. However, there is considerable heterogeneity. Localized segments of hypertrophy can occur anywhere including the apex which was original described in Japanese patients (Sakamoto *et al.* 1976) but is known to occur, all be it, less frequently in other populations. (Kitaoka *et al.* 2003) Hypertrophy can also be symmetrical or affect the mid-cavity at the level of the papillary muscles. (Poetter *et al.* 1996)

The degree of LV hypertrophy is also variable and can range from mild (13-15mm) to severe (>30mm) [Normal <13mm]. Extreme athletes can develop mild localised wall thickness creating diagnostic difficulty that can often only be resolved by a period of de-training and exclusion of abnormal relaxation.

LV hypertrophy is a physiologic response to increased afterload, volume overload, or acute or chronic ventricular injury in a variety of cardiac disease states. The increased LV wall thickness reduces ventricular wall tension (Laplace law: Tension = Pressure × LV radius ÷ 2LV wall thickness) and augments LV systolic function in the short and medium term. If hypertrophy continues to adequately compensate for increased wall stress then systolic function remains preserved.

However if hypertrophy fails to keep pace with wall stress then as the result of myocyte necrosis, fibrosis, and myocellular energy depletion, systolic dysfunction results and congestive cardiac failure ensues.

There is increasing evidence that the LV hypertrophy in HCM is also secondary, and may be an attempt to compensate for intrinsically impaired LV contraction. The primary abnormality in many instances may be malfunctioning of a sarcomeric protein. (Marian 2000) There is, for example, evidence that in HCM caused by missense mutations of the β-myosin heavy chain gene, mutant β-myosin translocates fluorescently-labelled actin at increased velocity in an *in-vitro* motility assay. (Cuda *et al.* 1997) In addition, single skinned slow skeletal (soleus muscle) myofibers, demonstrated to contain mutant β-myosin, generate reduced isometric force, unloaded shortening velocity and power. (Malinchik *et al.* 1997)

Thus, in contrast to cardiac conditions associated with increased afterload, such as hypertension, in HCM, normal systemic pressures are perceived as excessive load by a diseased myocardium with impaired contractile properties. Furthermore, as the hypertrophy and impairment of contraction are not uniform throughout the LV, regional differences in LV contractility may act as a stimulus for maintenance of the LV hypertrophy.

An important clinical feature of HCM is the presence or absence of obstruction to left ventricular outflow. Although it is only present in about 25-30% of patients (Maron *et al.* 2003c) it has lead to a confusing array of terms used to describe the condition, including idiopathic subaortic stenosis (Braunwald *et al.* 1964) and hypertrophic obstructive cardiomyopathy. However, HCM is preferred as it covers the whole spectrum of disease. Subaortic obstruction occurs as a result of

systolic anterior motion (SAM) of the mitral valve leaflets with mid-systolic contact of the leaflets with the ventricular septum. This results in physical obstruction to outflow with a higher proportion of forward flow being ejected in early systole. (Wilson *et al.* 1967) The exact cause of SAM is uncertain but maybe due to a drag effect created by the high velocity ejection jet (Sherrid *et al.* 1993; Sherrid *et al.* 2000) and/or the Venturi phenomenon. (Cape *et al.* 1989; Wigle *et al.* 1985)

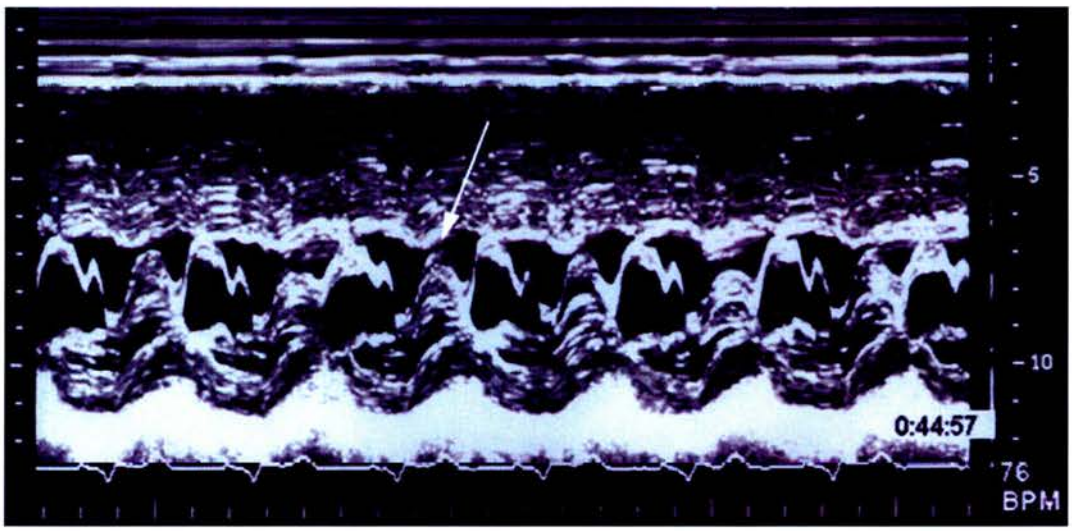

Figure 1-2: M-mode echocardiogram from patient with obstructive HCM demonstrating SAM of the mitral valve leaflets (arrow) with brief contact of the leaflets with the ventricular septum

The outflow tract obstruction results in a harsh crescendo-decrescendo murmur at the apex radiating to the base. In addition, a pansystolic murmur radiating to the axilla may be heard, as a result of the concomitant mitral regurgitation. There is often a bifid (not bisferiens) pulse and a double apex beat with the first impulse arising from early systole before the dynamic obstruction develops and a second impulse after.

Obstruction can also occur in the mid-cavity at the level of the papillary muscles, in which instance there is no SAM. (Wigle *et al.* 1985) This can be

associated with a distal LV aneurysm with VT arising from a substrate in the apical aneurysmal segment. In this case there is an hourglass configuration of the LV on echocardiographic four chamber views and LV ventriculography. **Figure 1-3.**

Figure 1-3: LV angiogram from patient with mid-cavity obstruction demonstrating a large apical aneurysm

Although there is a poor correlation between the magnitude of LV hypertrophy and reported symptoms in HCM, LV hypertrophy does have a direct relationship with prognosis. There is an increased risk of sudden death in patients with severe LV hypertrophy. (Elliott *et al.* 2000; Elliott *et al.* 2001; Spirito *et al.* 2000; Wigle *et al.* 1985) However, a small number of families with rare mutations in either troponin T or α-tropomyosin are also at increased risk of sudden death despite only mild LV hypertrophy. (Karibe *et al.* 2001; Varnava *et al.* 2001)

1.2. Diastolic Dysfunction

Diastolic dysfunction, even in the presence of normal systolic function, is believed to be responsible for functional limitation in a variety of cardiac disease states, although it is a difficult concept to define and the mechanisms by which this occurs is unclear. Diastole is traditionally defined as the period between the end of aortic ejection and the beginning of ventricular contraction of the succeeding beat. It is determined by both passive and active events. There is assumed to be passive relaxation of the myocardium from stored potential energy as well as active relaxation (inactivation) following re-uptake of cytosolic calcium by the sarcoplasmic reticulum.

A common haemodynamic abnormality in HCM is an elevated LV end-diastolic pressure (LV EDP) in the presence of normal or reduced LV diastolic volume. Hence, it is probably related to an alteration in the LV diastolic properties with a direct shift of the diastolic pressure-volume relation and impaired LV relaxation. The increased LV stiffness and impaired relaxation both result in elevated filling pressures and symptoms associated with cardiac failure.

The causes of impairment of passive relaxation in HCM include the degree of LV hypertrophy, fibrosis (accumulation of fibrillar Type I collagen, and an increase of the collagen I:III ratio) (Boerrigter *et al.* 1998; Lombardi *et al.* 2003; Mundhenke *et al.* 2002) and cellular disarray which are pathogonomic of the disease. (Davies and McKenna 1995; Hughes 2004)

Relaxation in HCM, as in coronary artery disease, is governed by inactivation-dependent mechanisms. Myocardial inactivation is an energy requiring process during which calcium (Ca^{2+}) is sequestered by the sarcoplasmic reticulum

(SR). In HCM, as in other forms of heart failure, there is down regulation of the SR Ca^{2+}-ATPase pump, resulting in persistent elevation of intracellular calcium concentration and prolonged interaction of contractile elements. (Gwathmey *et al.* 1991; Somura *et al.* 2001)

Exercise tolerance is dependent on an increase in cardiac output which in turn is dependent on heart rate and stroke volume augmentation by increased LV end-diastolic diameter and contractility. In HCM stroke volume augmentation is limited by the diastolic filling characteristics of the abnormal left ventricle and consequently exercise capacity is impaired. (Lele *et al.* 1995; Nihoyannopoulos *et al.* 1992)

Numerous studies have suggested the benefit of pharmacologic agents in the treatment of diastolic dysfunction in HCM. Improvement has been demonstrated with β-blockers, calcium antagonists (verapamil, diltiazem, and dihydropyridines), class 1A anti-arrhythmics (disopyramide, cibenzoline) and nicorandil. (Betocchi *et al.* 1996; Bonow *et al.* 1985; Bourmayan *et al.* 1985; Hamada *et al.* 2001; Izawa *et al.* 2003; Matsubara *et al.* 1995) Improved filling may also lead to enhanced exercise tolerance. However, these agents are vasodilatory and therefore will reduce afterload which may provoke or worsen outflow tract obstruction.

1.3. Myocardial Ischaemia

Myocardial ischaemia is common in patients with HCM despite angiographically normal coronary arteries, and consequently angina is a common symptom. (Lazzeroni *et al.* 1997) Regional myocardial perfusion abnormalities are demonstrated by exercise thallium scintigraphy and are often associated with apparent cavity dilatation indicative of sub-endocardial ischemia. (Cannon, III *et al.*

1991) In young patients, the myocardial ischemia is often not associated with chest pain, but is an important cause of symptoms of exercise-induced impaired consciousness. (Dilsizian *et al.* 1993)

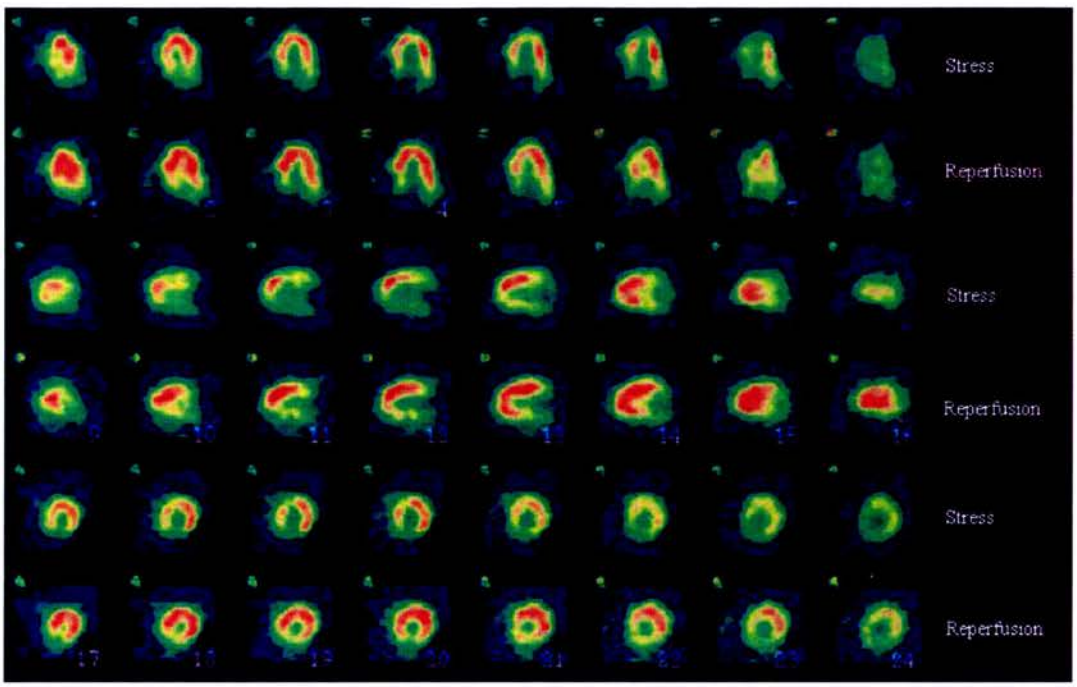

Figure 1-4: Thallium perfusion scan from patient with HCM. The top row from each slice is following stress. In addition to regional perfusion defects, there is apparent cavity dilatation indicative of sub-endocardial ischemia

Treatment of angina in HCM can be difficult as symptoms are often atypical and may not be related to exertion. β-blocker therapy and calcium antagonist are the mainstay of treatment for patients with HCM although no consensus exists about which drug to use first. (Braunwald *et al.* 2002; Cohen and Braunwald 1967; Goodwin 1973; McKenna and Behr 2002) Verapamil is the most commonly used calcium antagonist and is often effective in patients who fail to respond to β-blocker therapy. It also has been shown to cause reversal of the myocardial perfusion defects. (Petkow *et al.* 2000)

Ischaemia in HCM is multifactorial and is due to mechanisms which cause an imbalance between myocardial oxygen demand and myocardial perfusion. Increased demand occurs as a result of an increased muscle mass and an increase in both systolic and diastolic wall stress. The presence of outflow tract obstruction will further increase these wall stresses and exacerbate myocardial ischaemia. (Cannon, III *et al.* 1987) Relief of outflow tract obstruction reduces wall stress, improves haemodynamics and reduces the burden of myocardial ischaemia. (Cannon, III *et al.* 1991; Cannon, III *et al.* 1992)

Endothelial and smooth muscle intimal proliferation may result in luminal narrowing of the intra-myocardial small vessels and therefore decrease myocardial perfusion and is regarded as a form of 'small vessel' disease. (Maron *et al.* 1986b; Tanaka *et al.* 1987) These microvasculature abnormalities, together with the supply: demand mismatch may explain the impaired coronary vasodilator reserve that is observed in patients with HCM. (Cannon, III *et al.* 1985; Krams *et al.* 1998)

Bridging of an epicardial coronary artery by the myocardium is characterized by systolic compression and remains clinically silent in the vast majority of cases, but has been associated with myocardial ischaemia. Its reported prevalence is highly variable and is found, not surprisingly, more commonly at post-mortem than on angiography. (Mohlenkamp *et al.* 2002) It is thought to be more common in patients with HCM (Achrafi 1992) and is also associated with an increased risk of sudden cardiac death in children. (Yetman *et al.* 1998) This retrospective study included a highly select group of children undergoing coronary angiography and the association has been disputed. (Mohiddin *et al.* 1999; Mohiddin *et al.* 2000)

1.4. Arrhythmias

Supraventricular and ventricular arrhythmias occur commonly in patients with HCM. (Cecchi *et al.* 1995; Maron *et al.* 1999b) Atrial fibrillation is the most common sustained arrhythmia and occurs at some point in about 20-25% of patients (Olivotto *et al.* 2001; Robinson *et al.* 1990) and, as in the general population, has an increasing incidence with advanced age. Although AF can be associated with significant morbidity and mortality due to functional disability, heart failure and heart failure-related death, and embolic cerebrovascular disease, it is well tolerated in about one third of patients. (Olivotto *et al.* 2001) Heart failure is particularly associated with AF when onset occurs before age 50 years and is associated with outflow tract obstruction. (Olivotto *et al.* 2001) Embolic stroke has an incidence of about 1% and prevalence of 6% which is markedly increased compared to HCM patients without AF. (Furlan *et al.* 1984; Higashikawa *et al.* 1997; Maron *et al.* 2002; Robinson *et al.* 1990) Older age, heart failure, and left atrial size are significantly associated with an increased risk of thrombo-embolic events. (Maron *et al.* 2002)

The sudden onset of AF, resulting in loss of the atrial transport mechanism and irregular filling and emptying of the left ventricle, can cause devastating haemodynamic deterioration, and precipitate syncope or even SCD due to worsening of myocardial ischemia or precipitation of ventricular arrhythmias. (Boriani *et al.* 2002; Doi and Kitaoka 2001; Favale *et al.* 2003; Lopez *et al.* 2000; Olivotto *et al.* 2001)

One alternative therapeutic approach if anti-arrhythmic drugs are poorly tolerated is to perform AV nodal radio-frequency ablation with insertion of a permanent pacemaker. If the AF is paroxysmal, a dual chamber device with mode

switching capability can be implanted allowing the patient to take advantage of periods of sinus rhythm, while reverting to ventricular pacing mode during AF.

In addition to AF, patients can also be affected by other SVTs which are common in the general population, such as AV nodal and AV re-entry tachycardia. The latter has been described in association with HCM with families identified with the clinical syndrome of cardiac hypertrophy, AV conduction block and, accessory AV conduction. (Boriani *et al.* 2002; Gulotta *et al.* 1977) This familial condition was linked to a locus on chromosome 7q3 (7q34-q36) (MacRae *et al.* 1995) and was eventually identified to be a mutation in the gene for the γ2 regulatory subunit of AMP-activated protein kinase (*PRKAG2*). (Gollob *et al.* 2001) AMP-activated protein kinase acts as a metabolic sensor in cells. Disruption of the regulatory subunit *PRKAG2* may affect the normal regression of muscle fibres during AV septation. However, subsequent studies have shown that the cardiac hypertrophy differs significantly to that observed in HCM. There is certainly myocyte hypertrophy and a slight increase in interstitial fibrosis but there is no myofibrillar disarray which is the hall mark of HCM. In addition there is vacuole formation within the myocytes and evidence suggests these are filled with glycogen-associated particles. (Arad *et al.* 2002) Therefore the cardiac hypertrophy in these patients should really be considered a separate entity to HCM as it is more akin to a glycogen storage disease.

VT is also commonly observed in patients with HCM and recordings from ICDs at times of appropriate device therapy would suggest that ventricular arrhythmias are the commonest cause of SCD in HCM. Non-sustained VT is frequently seen on Holter monitoring and has been associated with an increased risk of SCD. (Maron *et al.* 1981; McKenna *et al.* 1981; Monserrat *et al.* 2003; Spirito *et*

al. 1994) Similarly VT induced during programmed ventricular stimulation at EP studies has been associated with an increased risk of SCD and has been used as an indication for implantation of an ICD. (Fananapazir *et al.* 1992) However, this has been strongly debated as the frequent occurrence of non-specific endpoints, such as polymorphic VT or VF has meant that invasive EP studies are thought to add little to the risk stratification of patients with HCM. (Behr *et al.* 2002) Some refinement of the EP protocol demonstrated an association between VF and increased fractionation of paced right ventricular electrograms in patients with HCM. (Saumarez *et al.* 1992) EP studies do still have a role in the assessment and treatment of other abnormalities such as the presence of accessory AV conduction pathways, atrial flutter or AF and occasionally monomorphic ventricular tachycardia which is often associated with mid-cavity obstructive HCM and a distal LV aneurysm. (Alfonso *et al.* 1989)

1.4.1. Sudden Cardiac Death

Although ventricular arrhythmias are likely to be the commonest cause of SCD in HCM, the mechanisms leading to this catastrophic event take account of a combination of factors. (Elliott *et al.* 2000) A large number of clinical features have been associated with an increased risk of SCD but they are all limited by poor positive predicative value. (Maki *et al.* 1998; Maron *et al.* 1981; McKenna *et al.* 1981; McKenna *et al.* 2002; Olivotto *et al.* 1999; Sadoul *et al.* 1997; Spirito and Maron 1990)

An abnormal blood pressure response to exercise, defined as a failure to augment blood pressure by >20 mm Hg during a maximum symptom-limited exercise test, gives a sensitivity of 75%, specificity of 66%, negative predictive value of 97% and positive predictive value of 15% in predicting SCD. (Sadoul *et al.* 1997)

This allows identification of patients who are at low risk of SCD but an abnormal blood pressure response has limited applicability in determining high risk patients without further risk stratification. Elliott et al further defined the criteria for an abnormal blood pressure response to exercise as failure to augment blood pressure by >25 mm Hg. In this study, there was a significant association between an abnormal blood pressure response and SCD in patients ≤40 years old but not in older patients. (Elliott *et al.* 2000)

The degree of hypertrophy in HCM has also been identified as a risk factor. A maximum wall thickness >30 mm has been associated with increased risk of SCD. (Elliott *et al.* 2000; Spirito and Maron 1990; Spirito *et al.* 2000) However this has also been contested as most SCDs in HCM occur in patients with maximum wall thicknesses less than 30 mm. (Elliott *et al.* 2001) In other studies no relationship between extent of LV hypertrophy and SCD could be found at all. (Olivotto *et al.* 2003)

The presence of non-sustained VT on Holter monitoring has probably received the most attention of all risk factors associated with HCM. (McKenna *et al.* 1981; Fananapazir *et al.* 1992; Maron *et al.* 1981; Monserrat *et al.* 2003; Spirito *et al.* 1994) However, the presence of non-sustained VT on ambulatory monitoring in HCM is extremely common. On routine monitoring, 90% of adults demonstrated ventricular arrhythmias. (Maron *et al.* 2003a) Non-sustained VT may only be important when associated with symptoms or when it occurs frequently. (Spirito *et al.* 1994)

A family history of SCD when combined with a history of syncope does have a significant positive predictive value for SCD. (Elliott *et al.* 2000; McKenna *et al.* 1981; McKenna and Behr 2002)

SCD occurs most frequently in children and young adults although can occur at any age. (Maron *et al.* 2000a) HCM is, in fact the most common cause of SCD in the young.

Much has been made of so called 'malignant mutations'. Since the discovery of the first gene mutation responsible for HCM, there has been hope that phenotype-genotype correlations would allow accurate prediction risk of SCD. However, as with most aspects of this heterogeneous disease, this too has proved difficult but genotyping may be included as part of a risk profile. (Firoozi *et al.* 2003; McKenna *et al.* 2002) It is certainly true that some gene mutations are associated with a higher incidence of SCD. (Epstein *et al.* 1992; Marian *et al.* 1995; Yamauchi-Takihara *et al.* 1996) Some mutations are associated with a high incidence of SCD but only mild hypertrophy. (Karibe *et al.* 2001; Watkins *et al.* 1995) For instance troponin T mutations have a high incidence of SCD with minimal or no hypertrophy but do have other hall marks of HCM such as myocyte disarray. (Varnava *et al.* 2001)

Perhaps one of the most controversial and difficult aspects of risk stratification in HCM is the risk attributable to exercise. Although SCD occurs most commonly during fairly mild exertion and occasionally even at rest, it is associated with more vigorous activities. (Maron *et al.* 1982; Maron *et al.* 2000a) This has lead to recommendations that athletes diagnosed with HCM should not continue participation often with significant psychological and even financial impact. Recent recommendations suggest that patients should not participate in competitive activities

at all, unless of particularly low intensity. However, an exception is made for older subjects provided they have no other significant risk factors for sudden death. (Firoozi *et al.* 2003) The American Heart Association defines various sporting activities depending on the perceived intensity. Recommendations are then made for different cardiovascular diseases as to which activities are discouraged and which should be permitted. The proviso, however, is that other clinical information is taken into account before a final decision is made. (Maron *et al.* 2003b)

The natural history of HCM and the incidence of SCD have been altered little by the use of anti-arrhythmic medications. Verapamil and β-blockers are largely ineffective in reducing the burden of ventricular arrhythmias and reducing the incidence of SCD. Amiodarone has been shown to reduce the frequency of ventricular arrhythmias in patients with HCM. (McKenna *et al.* 1984; McKenna *et al.* 1985) However, the ability to translate this reduction in ventricular arrhythmias into increased survival has been disputed. (Fananapazir *et al.* 1991; Gilligan *et al.* 1991) Amiodarone can be used successfully in the treatment of supraventricular arrhythmias, (Robinson *et al.* 1990) in particular AF. (Maron *et al.* 2002)

Improvement in ICD technology in recent years has led to an alternative treatment to anti-arrhythmic drugs for patients at high risk for SCD. ICDs have proved very effective in the prevention of SCD in a variety of cardiovascular disease states including HCM. (Maron *et al.* 2000b) Patients who have survived a cardiac arrest or sustained ventricular tachycardia are at particular risk of recurrent events and should therefore be considered for implantation of an ICD. (Elliott *et al.* 1999b) Retrospective studies of patients with ICDs have also shown clear benefit in patients who have them implanted for a secondary indication (previous cardiac arrest or

sustained ventricular tachycardia). (Begley *et al.* 2003) ICD implantation for primary prevention of SCD in HCM is less well defined. There are still significant device related complications and inappropriate shocks that limit their utility. Current guidelines for implantation of ICDs in HCM for primary prevention are classified as a class IIb indication (situations for which ICDs are frequently used but there is a divergence of opinion with respect to the necessity of implantation – usefulness/efficacy is less well established by evidence and opinion), and for secondary prevention as a class I indication (situations for which there is evidence and/or general agreement in favour of implantation).

1.5. Genetics

HCM has marked allelic variability with at least ten genes already identified as causing the disease. These ten genes are:

- β-myosin heavy chain - *MYH7*, 14q12 (Geisterfer-Lowrance *et al.* 1990)
- α-tropomyosin - *TPM1*, 15q22.1 (Thierfelder *et al.* 1994)
- troponin T - *TNNT2*, 1q32 (Thierfelder *et al.* 1994)
- myosin binding protein-C - *MYBPC3*, 11p11.2 (Bonne *et al.* 1995)
- essential and regulatory light chains of myosin - *MYL3*, 3p21.3-p21.2 and *MYL2*, 12q23-q24.3 (Poetter *et al.* 1996)
- troponin I - *TNNI3*, 19q13.4 (Kimura *et al.* 1997)
- titin - *TTN*, 2q24.3 (Satoh *et al.* 1999)
- cardiac actin - *ACTC*, 15q11-q14 (Olson *et al.* 2000)
- troponin C - *TNNC1* (Hoffmann *et al.* 2001)

HCM has also been associated with the Wolf-Parkinson-White syndrome and located to a further locus at 7q3 (MacRae *et al.* 1995). However, as already discussed, identification of the responsible gene (γ2 regulatory subunit of AMP-activated protein kinase *PRKAG2*) (Gollob *et al.* 2001), and additional histological evidence (Arad *et al.* 2002) has allowed reclassification of the hypertrophic component of this association as a glycogen storage disease distinct from other forms of HCM. (Arad *et al.* 2005) A further locus on chromosome 7 has also recently been identified. (Song *et al.* 2006) In addition considerable non-allelic heterogeneity exists with over one hundred mutations reported in *MYH7* alone.

These ten genes, however, barely account for 50% of patients with HCM. The remainder must have, as yet, undefined mutations in one of these genes or very likely in entirely different genes which may include the possibility of involving non-sarcomeric genes. Genetic screening of known genes in unrelated patients often yields previously unreported mutations and even, on occasion, more than one mutation in the same individual. These patients can be double heterozygotes or even homozygotes. (Mohiddin *et al.* 2003) Since the discovery of β-MHC mutations as a cause for HCM, HCM has been considered to be a 'disease of the sarcomere'. (Thierfelder *et al.* 1994) *PRKAG2* was therefore, initially thought to be the first non-sarcomeric gene mutation causing HCM. However, as indicated, recent evidence would suggest it is classified as a separate disease.

The mechanism by which sarcomeric gene mutations cause HCM, in particular the non-uniform pattern of hypertrophy is unfortunately unclear. It is incorrect to say that mutations result in weaker contractile sarcomeric units resulting in compensatory hypertrophy. Comparison of mutant and normal myosin

demonstrates enhanced kinetics of the cross-bridge cycle without affecting either the motion developed or the force generated. (Palmiter *et al.* 2000; Thierfelder *et al.* 1994) It must therefore be the random combination of mutant and normal protein that results in *abnormal* sarcomeric function. Increased stress perceived by the cytoskeleton will trigger compensatory mechanisms with local production of mitogens leading to hypertrophy.

Although as a general rule mutations in genes encoding proteins involved in force generation were associated with HCM and those involved in force transmission were associated with dilated cardiomyopathy, subsequent mutations have been found in genes that can be responsible for both HCM and dilated cardiomyopathy, such as *ACTC, TNNT* and even *MYH7*. (Kamisago *et al.* 2000; Olson *et al.* 1998; Stefanelli *et al.* 2004) This includes mutations that are found in the same functional domain of the subsequent protein.

1.5.1. Penetrance

To further complicate the situation, in addition to this marked genetic heterogeneity, extensive screening of families with known disease causing mutations have demonstrated that LV hypertrophy varies markedly even between family members with identical disease causing mutations (i.e. same genetic background). In some instances the degree of hypertrophy is undetectable and therefore the mutation is incompletely penetrant and patients are considered skips. The $908^{\text{Val-Leu}}$ and $256^{\text{Gly-Glu}}$ mutations in the *MYH7* gene are associated with ~60% penetrance, whereas, in contrast, the $403^{\text{Arg-Gln}}$ mutation in the *MYH7* gene is almost 100% penetrant. (Epstein *et al.* 1992; Fananapazir and Epstein 1994) **Figure 1-5**

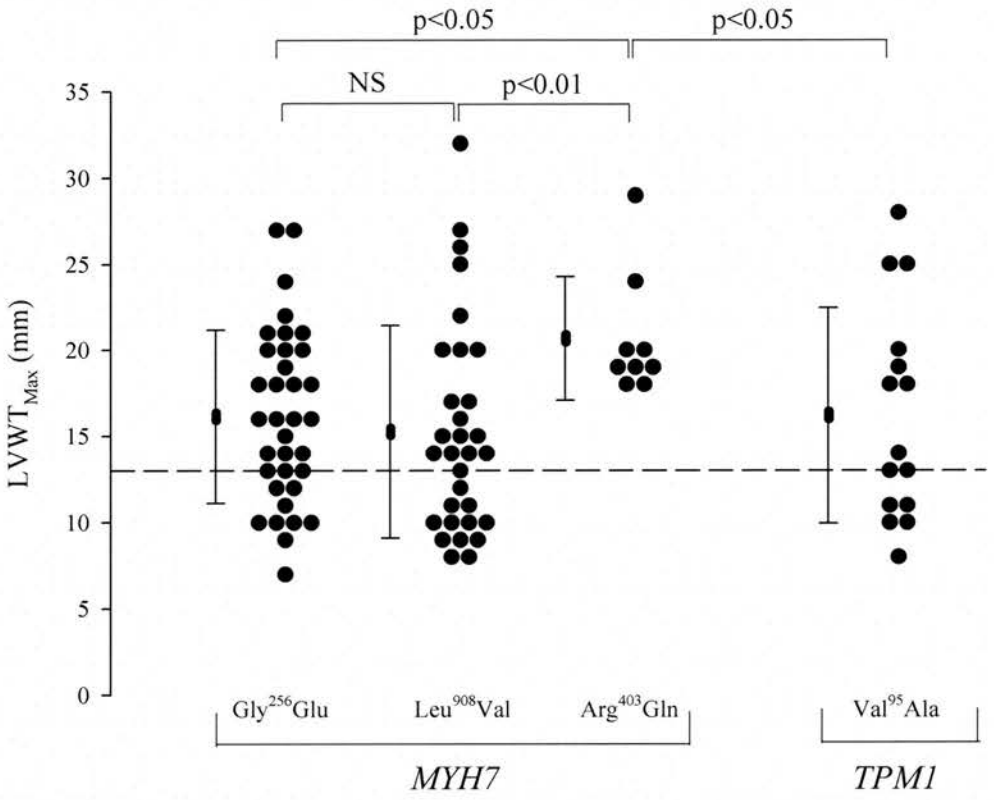

Figure 1-5: Variation in $LVWT_{max}$ between different mutations

Although the majority of HCM patients will have developed their full phenotype by the time of adulthood, delayed expression can occur. This is most evident in patients with mutations in the gene *MYBPC3*. (Niimura *et al.* 1998) This adds to the difficulty in screening family members as it is not possible to exclude the subsequent development of disease in adults without LV hypertrophy.

1.5.2. Phenotypic Variability

Some of the phenotypic variation observed is also mutation specific. The most important feature that is often attributed to specific mutations is prognosis. This has lead to certain mutations being considered 'malignant' while others are deemed 'benign' depending on the perception of risk of SCD. The *MYH7* mutation $403^{Arg\text{-}Gln}$ is almost universally accepted as malignant with high penetrance and incidence of SCD. (Epstein *et al.* 1992; Epstein *et al.* 1992; Watkins *et al.* 1992) Conversely there is not only low penetrance in the *MYH7* mutations $908^{Val\text{-}Leu}$ and $256^{Gly\text{-}Glu}$ but there is also a very low incidence of SCD. (Fananapazir and Epstein 1994) Still further mutations have a mild cardiac phenotype but a high incidence of SCD. (Karibe *et al.* 2001; Varnava *et al.* 2001) Other features have also been attributed to certain genes or mutations. Mutations in the essential and regulatory light chains are associated with a rare form of the cardiomyopathy where the hypertrophy is localised to the mid-cavity at the level of the papillary muscles resulting in a mid-cavity gradient rather than an outflow tract gradient. (Poetter *et al.* 1996) This form of HCM may be associated with a distal LV aneurysm and patients may be prone to monomorphic VT arising from the apical aneurysm.

Although much has been made of phenotype-genotype correlations there is, of course, a certain amount of disagreement. For instance the *MYH7* mutation $606^{Leu\text{-}Val}$ has variably been described as both malignant and benign. (Fananapazir and Epstein 1994; Watkins *et al.* 1992) Thus, distinct phenotypes in different families with the same underlying mutation highlight the influence of modifying factors, which may be genetic or non-genetic, in the phenotypic expression of HCM.

2. Methods

In Section II of thesis I have explored the role of four potential modifying factors in the phenotypic expression of HCM.

Gender has such an important impact on all cardiovascular disease it must be considered in addition when assessing any other modifying factors. I have therefore first considered the impact of gender on morphological development in HCM by comparing data from all HCM patients referred to National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health, Bethesda, Maryland, who had undergone cardiac MRI.

I also studied the role of IGF-I, a potent mitogen of cardiomyocytes, and its major plasma binding protein, IGFBP3, in influencing the development of LV hypertrophy in HCM. A small subset of these patients had levels of BNP measured to determine its relationship to disease severity and heart failure symptoms in HCM.

Finally common genetic polymorphisms in two genes (ACE and ET-1) that theoretically were felt likely to influence LV hypertrophy genesis in HCM were explored.

In Section III, following on from the observations from our genetic studies on the involvement of the renin-angiotensin system on LV hypertrophy in HCM, a randomised, double-blind placebo-controlled study was conducted to investigate the possibility of LV hypertrophy regression using ACE inhibitors and/or ARB drugs.

2.1. Patient Population

A total of 269 patients were included in all studies (235 adults, ≥20 years; 34 children, <20 years). To evaluate gender differences, 208 adult patients with morphologic evidence of HCM were compared. The influence of IGF-I and its major binding protein IGFBP3 on the development of LV hypertrophy in patients with known sarcomeric mutations was assessed in 100 patients (50 patients with morphologic evidence of HCM and 50 patients with a disease causing mutation but without LV hypertrophy) and 50 family members without disease causing mutation (controls). BNP levels were measured in 37 of these patients with a disease causing mutation and 19 controls. HCM genotyping had already been established in a number of large kindred in our own laboratory under separate genetic protocols. DNA from 229 patients was tested for genetic modifiers of HCM. ***Figure 2-1.***

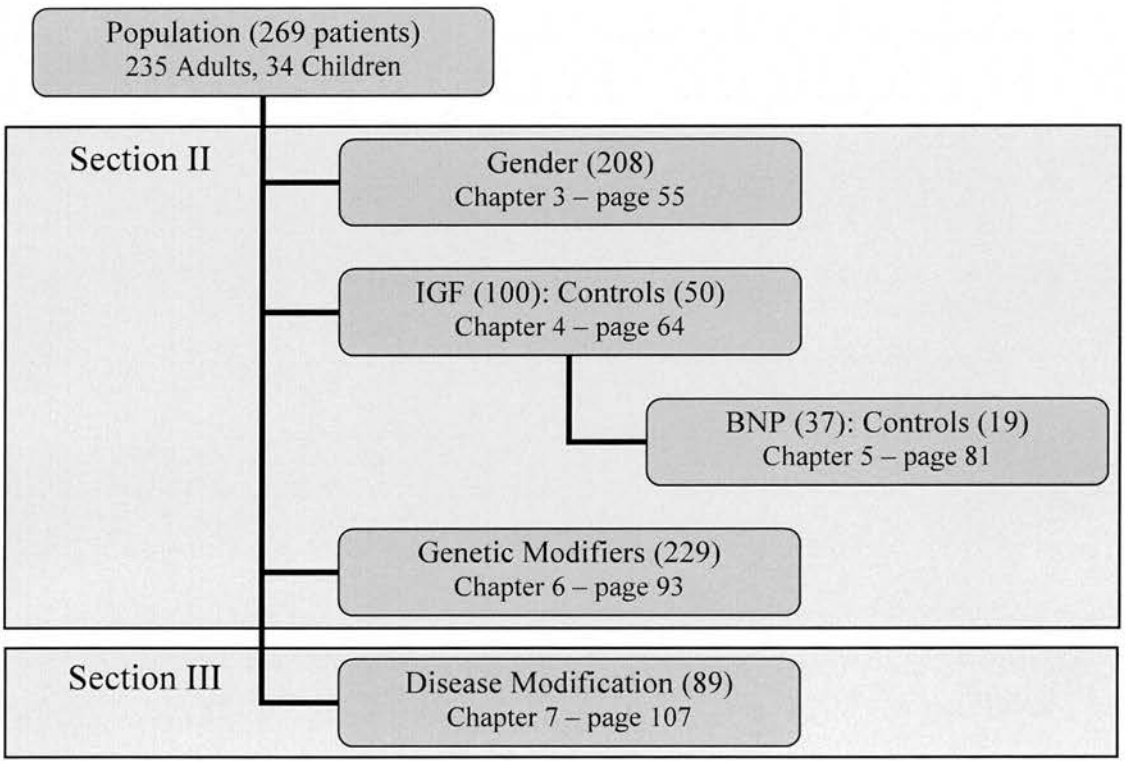

Figure 2-1: Distribution of patients included in studies

Eighty-nine patients were enrolled in the prospective randomised, double-blind, placebo controlled study examining LV hypertrophy regression in HCM.

Patients were referred to the NHLBI from centres mainly within the USA either having had the diagnosis of HCM established or for the purposes of screening. Patients could also self-refer and as a result some patients from other countries were included. In addition family screening was undertaken in patients with sufficiently large pedigrees, if requested, or were the disease causing mutation was known.

All HCM patients seen at NHLBI were consented with a screening protocol which permitted basic investigations to allow initial phenotyping. Patients and family members who participated in family screening studies signed a separate consent. In addition a separate consent for genetic studies was signed by anyone from whom DNA was collected. This permitted the analysis of DNA for mutations suspected of causing HCM as well as specific mutations suspected of being potential modifiers of the disease. Specific protocols and consents were obtained from patients enrolled in the studies looking at IGF-I in HCM and regression of LV hypertrophy with ACE inhibitors and ARB drugs. All protocols and consents were approved by the Institutional Review Board of the NHLBI.

HCM in adults was defined as a diastolic LV wall thickness of $\geq$13 mm by echocardiography (in children >2 standard deviations of published normal values) in the absence of another cause for the increased cardiac hypertrophy. Diastolic LV wall thickness of >16 mm by MRI was also considered to indicate hypertrophy This higher value was determined from our own studies on normal controls and is likely due to the greater spatial resolution of the MRI images. Other causes of the LV hypertrophy were excluded.

2.2. Investigations

2.2.1. Echocardiography

All patients underwent initial assessment by echocardiography. Standard transducer positions were used to obtain two-dimensional images. The distribution of LV hypertrophy was assessed in the parasternal long axis, short axis, and apical two and four chamber views. Continuous wave Doppler was used initially to determine the presence of outflow tract or intra-cavitary gradients. If a gradient was established, pulsed wave Doppler was used to determine the level of obstruction and to assist exclusion of coexistent aortic valve disease. A gradient of ≥30 mm Hg was considered significant obstruction.

2.2.2. Cardiac Magnetic Resonance Imaging

MRI was used to assess LV mass and volumes. Scanning was performed in a 1.5 T clinical scanner (GE, Waukesha, WI) using a cardiac phased array coil. The studies were gated to the ECG and acquired during a single breath hold. Contiguous multi-slice short axis images (8 mm thickness) were obtained to define the site of maximal LV wall thickness at end diastole ($LVWT_{max}$). Measurement of LV volumes, mass and ejection fraction were made offline on a dedicated workstation. The epicardial and endocardial borders of each short axis image at both end-diastole and end-systole were then traced by hand providing an end-diastolic (ED) and end-systolic (ES) area for each slice. As the slices were of known thickness, it was then possible to calculate ED and ES volumes for the LV cavity and myocardium for each slice. The summation of these volumes provided ED and ES LV volumes (EDV and

ESV) as well as the ED volume of LV myocardium. The volume of myocardium was then multiplied by an assumed specific gravity of cardiac muscle (1.05g/ml) to provide the ED LV mass. Stroke volume (SV) was calculated as the difference between EDV and ESV volume. The heart rate was recorded at the time of the study which allowed calculation of cardiac output (CO) from the derived SV (HR x SV). Ejection fraction (EF) was also calculated from the EDV and ESV (SV/EDV x 100). The LA dimension was measured from the four chamber view.

Validation of MRI Measurements: The ability of MRI to accurately determine volumes was first validated with the aid of water filled phantoms of varying sizes (range 7.5-465 ml). There proved to be excellent correlation between the volume of 15 water-filled phantoms and their absolute volume: $\text{Volume}_{\text{phantom}} = 1.00 \times \text{Volume}_{\text{absolute}} - 1.5$ ml, $R^2 = 0.9998$. In addition the inter-observer variability was determined for cardiac indices (normal subjects, and patients with coronary artery disease, HCM and valvular heart disease). ***Table 2-1 and Figure 2-2.***

Cardiac Parameter	n	Linear Relation	R^2	Co-efficient of Inter-observer Error
LV Volumes (ml)	20	y=1.01x+3.8	0.987	6.1%
LV end-diastolic mass (g)	9	y=0.996x+3.9	0.973	4.7%
LV end-systolic mass (g)	9	y=1.09x+21.3	0.989	4.1%
LV ejection fraction (%)	10	y=1.002x+0.42	0.916	5.9%
LV stroke volume (ml)	10	y=0.957x+5.7	0.992	3.7%
x and y, observer 1 and 2				

Table 2-1: Inter-observer variability of cardiac indices by MRI

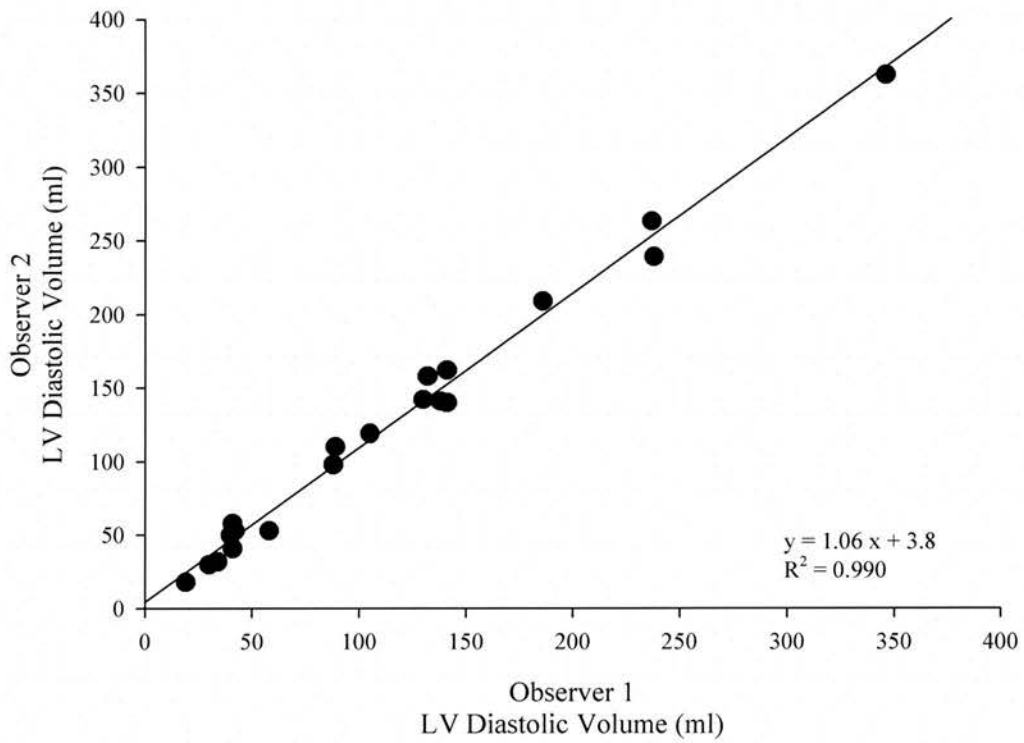

Figure 2-2: Correlation of LV diastolic volume between 2 observers

LV mass should not change throughout the cardiac cycle. Although the LV thickens during systole, the length of the ventricle shortens. Therefore the volume of LV myocardium and hence LV mass should be the same in systole and diastole. These values were therefore compared as an additional internal measure of validity. ***Figure 2-3.***

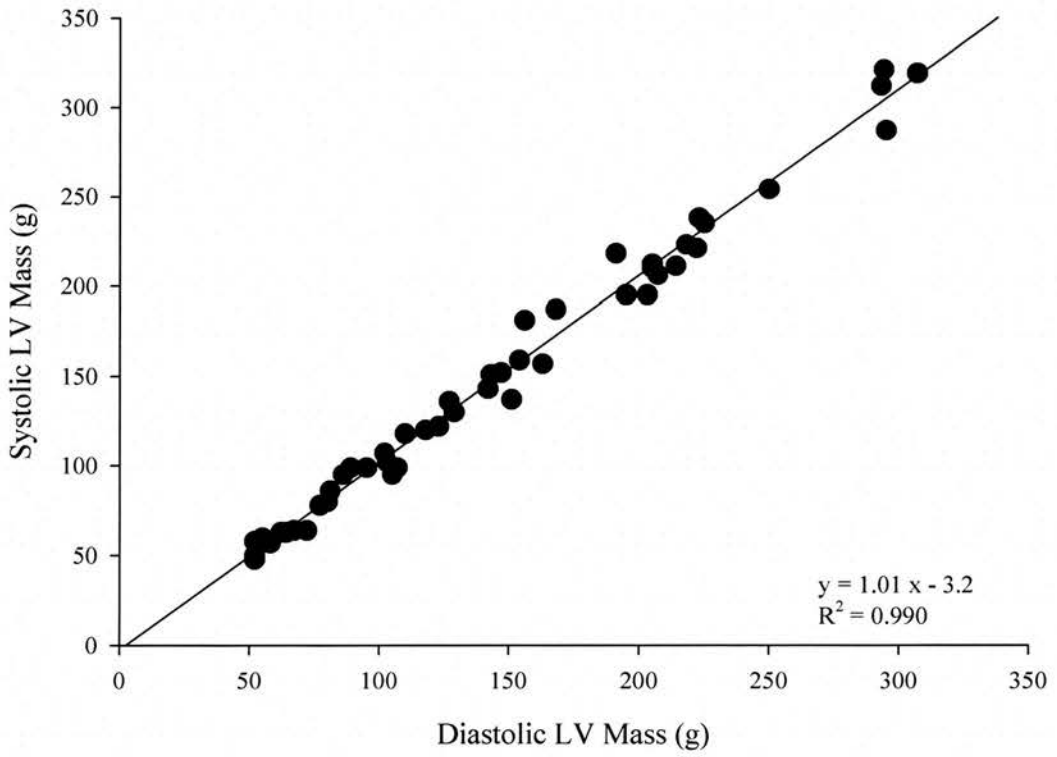

Figure 2-3: Correlation between systolic and diastolic LV mass

LV ejection fraction (EF) measured by MRI was compared to LV EF measured by radionuclide angiography (RNA) in 46 subjects. The relation was best described by the following quadratic equation: $EF_{MRI} = -0.0058x(EF_{RNA})^2 + 1.391x(EF_{RNA})$, $R^2 = 0.8856$. The deviations from the quadratic fit and actual MRI measurements were relatively small but became larger in the range of 55 to 75%. In subjects with EF <70% (n=31) the relation was described by the following linear regression equation: $EF_{MRI} = 1.01x(EF_{RNA}) + 3.49$, $R^2 = 0.8535$. ***Figure 2-4.***

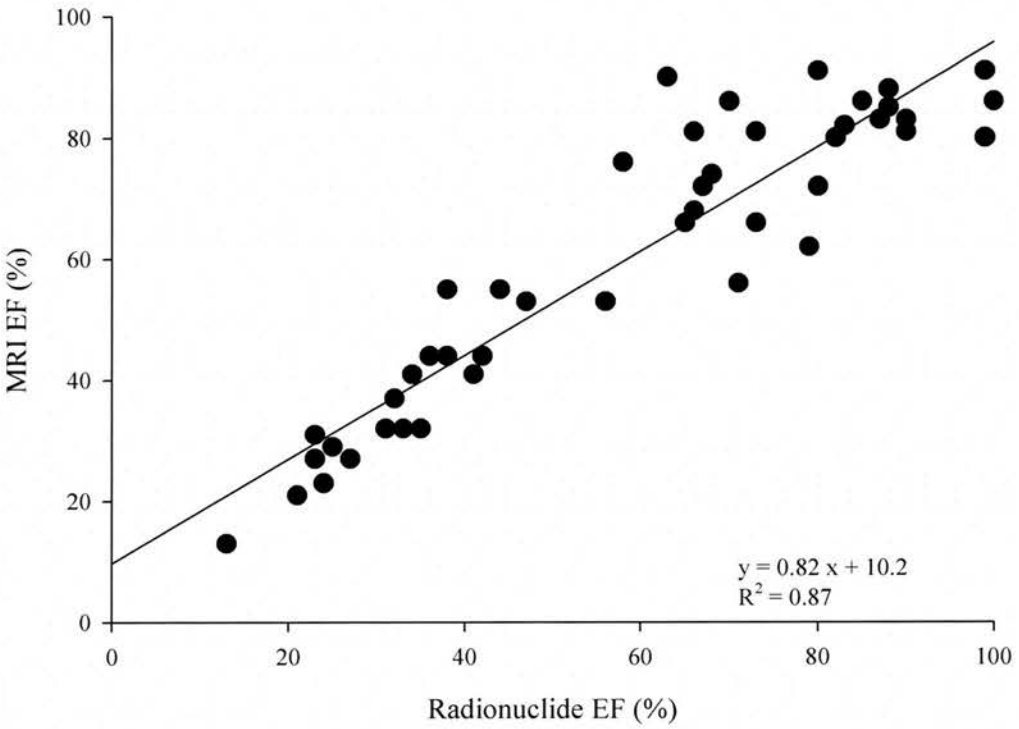

Figure 2-4: Correlation between EF measured by MRI and radionuclide angiography

SECTION II: MODIFYING FACTORS

3. Gender Differences in the Phenotypic Expression of Hypertrophic Cardiomyopathy

3.1. Introduction

Gender-specific cardiovascular differences have been described in normal humans and animals. Functional parameters, such CO and EF, are gender independent; whereas morphological parameters, LV volumes and mass, are gender dependent even after correction for body surface area. (Lorenz *et al.* 1999) Similar differences are observed for right ventricular parameters.(Forman *et al.* 1997; Lorenz *et al.* 1999; Marcus *et al.* 1999; Sandstede *et al.* 2000; de Simone G. *et al.* 1995)

In addition, the cardiac response to several cardiovascular disease states also demonstrates gender-specific differences. A greater degree of LV hypertrophy, smaller chamber volumes and consequently lower wall stress is observed in women with aortic stenosis compared to men. (Aurigemma and Gaasch 1995; Kostkiewicz *et al.* 1999; Marcus *et al.* 1999; Rohde *et al.* 1997) A similar pattern of gender difference is also observed in hypertension with a much stronger correlation between indices of LV hypertrophy and blood pressure observed in women. (Savage *et al.* 1998; Vriz *et al.* 1997; Zabalgoitia *et al.* 1996) In addition, experimental models of pressure overload hypertrophy not only demonstrate similar morphologic differences but suggest there is an earlier transition to heart failure in male animals. (Douglas *et al.* 1998; Weinberg *et al.* 1999)

Although a similar degree of LV hypertrophy is seen between male and female rats during chronic volume overload due to an aorto-caval fistula, LV

volumes are better preserved in the female rats, again limiting the increase in wall stress. (Gardner *et al.* 2002)

SCD is much more common in men compared to women, however, women have longer corrected QT intervals and a higher incidence of Torsades de Pointes. This apparent paradox is likely to be explained by the greater prevalence of coronary artery disease in men, although a higher proportion of SCD in women occurs in the absence of prior overt coronary artery disease. (Dahlberg 1990; Larsen and Kadish 1998)

The role of gender in determining cardiac phenotypic expression and clinical presentation in HCM has been uncertain, in part due to lack of a reliable method for measuring LV mass in HCM. Cardiac MRI provides a powerful tool to define potential differences in LV dimensions and function that are independent of geometry. Understanding the impact of gender on phenotypic expression is an important consideration before assessing the contribution of other potential modifiers.

3.2. Methods

Adult patients, who had been assessed as part of other studies, and who had morphological evidence of HCM, were included in this comparison. Two hundred and eight adult patients met the criteria for the diagnosis of HCM as detailed previously (Mean age 41±0.9 years, range 19-76 years). There were 116 male patients and 92 female patients. All patients had previously undergone a screening echocardiogram and cardiac MRI. Cardiac MRI parameters were compared to assess gender differences. Sixty-one patients (27 male and 34 female) had significant

outflow tract obstruction. Therefore the cardiac MRI parameters of the remaining non-obstructed patients were also compared separately to exclude the influence of obstruction on these parameters. All parameters were also compared after correction for BSA, calculated using the Dubois formula (BSA = 0.20247 x height (m) $^{0.725}$ x weight (kg) $^{0.425}$). (DuBois and Dubois 1916) The blood pressure documented on initial assessment was recorded and used for the purposes of analysis. The ratio of $LVWT_{max}$ to EDV is proportional to wall stress and was calculated for all patients and compared between groups.

3.2.1. Statistics

Comparisons between male and female patients were made with an unpaired student t-test. Analysis of co-variance was also performed to include other confounders. All results are expressed as mean ± SEM. A p-value < 0.05 was considered significant.

3.3. Results

Male and female patients (all patients and non-obstructed patients) were well matched for age. However, women had a significantly lower systolic and diastolic blood pressure in addition to lower height, weight and BSA. ***Table 3-1.***

	All patients		Non-obstructed	
	Male n=116	Female n=92	Male n=89	Female n=58
Age (years)	41±1.1	42±1.5	40±1.2	40±1.7
systolic BP (mm Hg)	125±1.5*	118±1.7	126±1.7†	117±2.3
diastolic BP (mm Hg)	75±1.0**	69±1.1	74±1.3††	67±1.3
mean BP (mm Hg)	92±1.0***	85±1.2	91±1.2†††	84±1.3
Height (m)	1.77±0.01***	1.63±0.01	1.77±0.01†††	1.63±0.01
Weight (kg)	87±1.4***	74±1.8	87±1.5†††	73±2.3
BSA (m^2)	2.04±0.02***	1.78±0.02	2.03±0.02†††	1.77±0.03
*p<0.001, **p<0.0005, p<0.0001, male vs. female (all patients); †p<0.005, ††p<0.0002, †††p<0.0001, male vs. female (non-obstructed)				

Table 3-1: Comparison of blood pressure, height, weight and BSA by gender

SV, EDV, ESV and LV mass were all lower in women compared to men. However there was no difference in EF or $LVWT_{max}$. The ratio $LVWT_{max}$: EDV was significantly higher in women compared to men. ***Table 3-2.***

	All Patients		Normal^	
	Male	Female	Male	Female
SV (ml)	91±2.6**	75±2.2		
EF (%)	69±0.9	72±1.0	67±0.7	67±0.9
EDV (ml)	123±3.1**	99±2.4	136±4.4	96±4.3
ESV (ml)	32±1.3**	25±1.1	45±2.0	32±1.7
LV mass (g)	241±8.9**	192±7.4	178±4.5	125±4.9
$LVWT_{max}$ (mm)	24±0.6	24±0.6		
$LVWT_{max}$/EDV (mm/ml)	0.21±0.01**	0.26±0.01		
*p<0.05, **p<0.0001, male vs. female; ^ Selected normal values derived from Lorenz et al, 1999				

Table 3-2: Comparison of MRI parameters between male and female patients

	Non-obstructed		Normal^	
	Male	Female	Male	Female
SV (ml)	88±3.0††	71±2.4		
EF (%)	68±1.0	70±1.3	67±0.7	67±0.9
EDV (ml)	121±3.6†††	97±2.7	136±4.4	96±4.3
ESV (ml)	33±1.5†	26±1.3	45±2.0	32±1.7
LV mass (g)	221±8.7†	173±8.7	178±4.5	125±4.9
$LVWT_{max}$ (mm)	23±0.6	23±0.6		
$LVWT_{max}$/EDV (mm/ml)	0.21±0.01†	0.25±0.01		

†p<0.0005, ††p<0.0002, p<0.0001, male vs. female; ^ Selected normal values derived from Lorenz et al, 1999

Table 3-3: Comparison of MRI parameters between non-obstructed male and female patients

After correction for BSA, differences still existed in EDV and ESV (not non-obstructed patients). However, there was no longer any significant difference in SV or LV mass. $LVWT_{max}$ when corrected for BSA was actually significantly greater in women compared to men. ***Table 3-4 and Table 3-5.***

	All Patients		Normal^	
	Male	Female	Male	Female
SV-I (ml/m^2)	44±1.2	42±1.0		
EDV-I (ml/m^2)	60±1.5*	56±1.2	69±1.6	61±1.9
ESV-I (ml/m^2)	16±0.6*	14±0.6		
LV mass-I (g/m^2)	118±4.3	108±4.2	91±1.6	79±1.5
$LVWT_{max}$-I (mm/m^2)	12.0±0.3**	13.6±0.4		

-I, indexed to BSA; *p<0.05, **p<0.001, male vs. female; ^ Selected normal values derived from Lorenz et al, 1999

Table 3-4: Comparison of MRI parameters correct for BSA between male and female patients

	Non-obstructed		Normal^	
	Male	Female	Male	Female
SV-I (ml/m^2)	43±1.4	39±1.1		
EDV-I (ml/m^2)	60±1.7†	55±1.3	69±1.6	61±1.9
ESV-I (ml/m^2)	16±0.7	15±0.8		
LV mass-I (g/m^2)	109±4.1	99±5.6	91±1.6	79±1.5
$LVWT_{max}$-I (mm/m^2)	11.6±0.3†	13.1±0.5		
†p<0.05, male vs. female; ^ Selected normal values derived from Lorenz et al, 1999				

Table 3-5: Comparison of MRI parameters corrected for BSA between non-obstructed male and female patients

To assess whether gender was independently predictive of LV mass and volumes in HCM, analysis of co-variance was performed on all patients including age and systolic BP as potential confounders. The percent of variance in $LVWT_{max}$-I explained by these variables was 16.1% ($p<0.0001$) and gender was an independent predictor ($p<0.005$). Gender was also an independent predictor of $LVWT_{max}$/EDV ($p<0.0005$). The percent of variance explained by the whole model was 10.0% ($p<0.0001$). There was no significant co-variance between the variables and SV-I, EF or LV mass-I but there was some co-variance between the variables and EDV-I and ESV-I ($R^2=5.2\%$, $p<0.02$ and $R^2=3.8\%$, $p<0.05$ respectively) with gender being an independent predictor in both instances ($p<0.005$ and $p<0.01$ respectively).

The same analyses were performed on the non-obstructed subset of patients. The percent of variance in $LVWT_{max}$-I was 17.5% ($p<0.0001$) and gender was an independent predictor ($p<0.05$). The percent of variance in $LVWT_{max}$/EDV was 11.36% ($p<0.001$) and again gender was an independent predictor ($p<0.005$). There was no significant co-variance between these variables and SV-I, EF, EDV-I, ESV-I or LV mass-I.

3.4. Discussion

Gender differences in LV hypertrophy have been reported in a variety of disease states. (Douglas *et al.* 1998; Kostkiewicz *et al.* 1999; Rohde *et al.* 1997; Savage *et al.* 1998; Vriz *et al.* 1997; Weinberg *et al.* 1999; Zabalgoitia *et al.* 1996) In animal studies, normal female rats had smaller indexed LV masses and smaller LV volumes but had thicker indexed walls. (Forman *et al.* 1997) This relationship was maintained in rats subjected to pressure overload by aortic banding. However, although the LV mass of female rats continued to rise with age, that of male rats fell with advancing age suggesting an early transition to dilatation and heart failure. (Douglas *et al.* 1998)

MRI studies of normal human volunteers showed greater LVM-I, EDV-I, ESV-I, and LVWT-I in men compared to women and this relationship did not alter with increasing age. (Sandstede *et al.* 2000) Rohde *et al* studied the gender differences in left ventricular geometry in relation to aortic valve disease and the effect of particular valve lesions. Female patients with pure aortic stenosis had smaller LV mass and volumes but greater wall thickness. Female patients with pure aortic regurgitation, however, had similar wall thickness but still had smaller LV mass and volumes. (Rohde *et al.* 1997) Similar observations have been made in adult patients with hypertension. (Vriz *et al.* 1997) Our results, suggest that the hypertrophic response in women and men with HCM is similar to that observed in adult patients with other forms of pressure overload.

Attempts to explore the impact of gender on phenotypic expression in patients with HCM have concluded that no difference exists in LV wall thickness, although women did have smaller LV mass and volumes compared to men.

(Dimitrow *et al.* 1998; Maron *et al.* 1999a) However in these studies men were compared directly to women with no attempt made to correct for the smaller body size of women and this may have concealed important differences in measured parameters.

These studies also used cardiac ultrasound to evaluate the gender differences. LV mass was derived indirectly from equations that make assumptions of LV morphology. These calculations probably do not apply to HCM as in HCM the cardiac hypertrophy is often asymmetric and regionally distributed resulting in distortion of the LV cavity. Further, the cardiac morphology varies markedly from one HCM patient to another. We therefore used MRI to study the cardiac effects of gender in HCM as it provides a more direct assessment of LV structure (mass and volumes) and function.

Although women in our study have smaller LVM-I, they have greater LVWT-I, which may represent favourable adaptation, as this combination of small, thick walled hearts will result in significantly reduced wall stress. Indeed, relative wall thickness (LVWT/EDV) was significantly greater in women compared to men. These results may therefore partially explain the survival advantage for women with HCM that has been reported previously. (Koga *et al.* 1984)

3.4.2. Limitations

A single definition of HCM is applied to both male and female patients. As body size clearly has an impact on cardiac dimensions, borderline cases may be reported as normal. Therefore a greater number of female patients may be diagnosed as normal or mistakenly considered phenotypically negative in the presence of a disease causing mutation. However, $LVWT_{max}$/EDV is internally controlled and

should largely be independent of overall body size. Although, BSA has a better correlation with cardiac dimensions than height or weight (Lorenz *et al.* 1999), it is uncertain whether this is the optimum method to correct these values for body size and as such, spurious differences may exist in indexed values. Information on medication use in these patients was not available.

3.5. Conclusions

Distinct gender-specific differences in cardiac morphology exist in patients with HCM. Women have smaller, thicker walled hearts compared to men. The pattern of gender differences is similar to that observed in patients with pressure overload but not other forms of hypertrophy indicating that the hypertrophic response in HCM may be due to a perceived pressure overload by abnormal sarcomeres. Although women have relatively thicker hearts, cavity size is smaller, which will reduce wall stress, and may provide a distinct survival advantage. These results have important implications for the design of studies that examine genes and therapies that may modify phenotypic expression in HCM.

4. Influence of Insulin-like Growth Factor-I and IGFBP3 on Phenotypic Expression in Hypertrophic Cardiomyopathy

4.1. Introduction

In this study we explore the ability of IGF-1 and IGFBP3 to modulate LV hypertrophy of HCM in a genetically-defined population. IGF-1 is an attractive candidate in this respect for several reasons. It mediates most of the actions of growth hormone. Receptors for IGF-1 are expressed in cardiac myocyte, and signal cell growth, differentiation and survival through endocrine, paracrine or autocrine mechanisms. (Fuller *et al.* 1991; Ito *et al.* 1993) They stimulate cardiomyocyte hypertrophy by increasing protein synthesis and accumulation (Decker *et al.* 1995; Fuller *et al.* 1991), retard apoptotic signalling (Chen *et al.* 2000; Morales *et al.* 2000; Sun *et al.* 2000), and improve cardiomyocyte function. (Kinugawa *et al.* 1999; Li *et al.* 1997a) Due to their inotropic effects, IGF-1 or GH have been used with variable success in the treatment of chronic heart failure. (King *et al.* 2001; Perrot *et al.* 2001; Welch *et al.* 2002) Further, high IGF-1 states such as acromegaly are associated with LV hypertrophy, and GH/IGF-1 suppression in affected patients is followed by regression of the LV hypertrophy and improved cardiac function. (Colao *et al.* 2001) Cardiac excitation-contraction coupling has been shown to be impaired in ventricular myocytes of transgenic animals with IGF-1 deficiency (Ren and Brown-Borg 2002), and in humans, low IGF-1 in GH deficiency syndrome is associated with reduced LV mass and impaired LV function, and GH replacement therapy normalizes these cardiac indices. (Colao *et al.* 2002) IGF-I gene expression is up-regulated in

hypertrophied myocardium, including HCM. (Li *et al.* 1998; Li *et al.* 1997b; Pauliks *et al.* 1999)

These findings have led to attempts to reverse the cardiac hypertrophy in HCM using specific inhibitors of GH/IGF-1 system. (Demirtas *et al.* 1998; Gunal *et al.* 1996) However, despite the significant advances in our understanding of IGF-1, its role in HCM remains uncertain. This study therefore examines whether differences in LV hypertrophy in HCM may be influenced by differences in circulating concentrations of IGF-I and IGFBP3.

4.2. Methods

The study was performed after informed consent and consisted of 150 subjects from 33 unrelated families (5±6 subjects per family) who were genotyped. (Fananapazir and Epstein 1994) One hundred subjects from several unrelated families had one or more of 26 disease mutations in one of six sarcomeric genes. ***Table 4-1.***

Subjects with positive genetic diagnosis were separated into two groups based on magnetic resonance estimations of $LVWT_{max}$: Group 1 were adult patients and young HCM patients (<20 years) with a $LVWT_{max}$ of >16 mm or >2 SD above published normal values, respectively; and Group 2 were subjects with $LVWT_{max}$ within the range of normal controls. The control group consisted of 50 family members without a disease causing mutation. Subjects also underwent echocardiography, treadmill and bicycle exercise tests, and ambulatory electrocardiographic (ECG) Holter monitoring.

Gene	Mutation	Group 1	Group 2	Total
MYH7	R143Q	0	1	1
	M149V	2	0	2
	Y162C	1	0	1
	N187K	3	3	6
	G256E	0	1	1
	G389E	3	3	6
	R403Q	5	1	6
	D479N	1	0	1
	V606W	0	1	1
	R663H	3	4	7
	R719W	3	0	3
	R723C	3	0	3
	E743D	1	0	1
	G768R	1	0	1
	R847H	3	3	6
	R870H	2	1	3
	D906G	0	5	5
	L908V	8	14	22
TPM1	V95A	5	5	10
ACTC	V9L	0	1	1
	G99K	3	0	3
MYL2	K111X	1	1	2
	P95R	1	0	1
MYL3	R154H	0	1	1
MYBP3	T842X	2	0	2
	E258K	3	1	4
Total		54	46	100

Table 4-Ì: Molecular defects causal for HCM

4.2.1. Plasma IGF-I and IGFBP3 Concentrations

Only a small amount of IGF-1 circulates in the free form. Most of the plasma IGF-1 is bound to specific carrier proteins, insulin-like growth factor binding proteins (IGFBPs). These have an important role in the regulation of IGF-1. IGFBP3 accounts for more than 90% of all circulating IGFBPs, although all six identified carrier proteins probably have distinct physiologic roles. (Kelley *et al.* 1996) Blood was obtained in the non-fasting state after one hour of supine rest. Serum or EDTA plasma sample was prepared for determination of IGF-I and IGFBP3 concentrations within one hour of blood collection and shipped frozen in a plastic vial to Endocrine Sciences, Calabasas Hills, CA 91301. The hormone concentrations are measured using radio-immunoassay (RIA) using a monoclonal antibody directed against a synthetic segment of the IGF-I peptide and a high affinity polyclonal antibody specific for the binding subunit, IGFBP-3β, of IGFBP3.

4.2.2. Statistics

Data are presented as mean ± 1 SEM. Differences between mean values were compared by one-way ANOVA. Bonferroni multiple comparisons test was used to test differences between individual groups. Spearman's correlation was used to examine the relation between two variables. A two-tailed $p<0.05$ was considered significant. To evaluate the relationship between IGF-1 and LVWT, we used covariance techniques that account for the familial relationships among the members of the study. A variance component analysis approach (Hopper and Mathews 1982) being implemented in the genetic software, SOLAR (Almasy and Blangero 1998) was used for the data analysis. This approach is able to deal with families of different

sizes and structures. It takes full account of the different types of relationship within a family in performing association studies on two quantitative traits.

4.3. Results

4.3.1. Magnetic Resonance Criteria of LV hypertrophy and HCM

Family members without genetic mutation (Controls): By MRI, $LVWT_{max}$ was $\leq$16 mm and LV mass was <170 g in all of the 23 normal adult subjects. Similarly, $LVWT_{max}$ was $\leq$14 mm and LV mass was <165 g in all of the 27 young control subjects.

Subjects with sarcomeric gene mutations: $LVWT_{max}$ varied markedly from 9 mm to 44 mm, and LV mass from 57 g to 670 g in the 68 adult subjects with positive genetic diagnosis. $LVWT_{max}$ also varied markedly from 9 mm to 28 mm, and LV mass from 37 g to 208 g in the young subjects with disease mutation. Although there was a significant correlation between $LVWT_{max}$ and LV mass, the same LV mass was associated with up to 20 mm difference in LV wall thickness. ***Figure 4-1.***

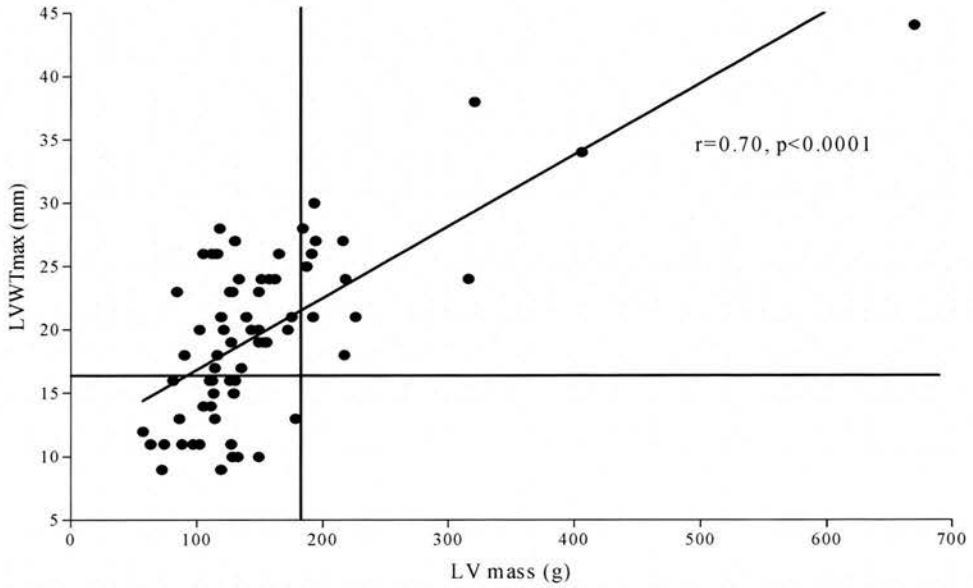

Figure 4-1: Relation between maximum LV wall thickness (LVWT) and LV mass in 68 adult subjects with disease mutation.

In this selected population, $LVWT_{max}$ was >16 mm (HCM present) in 44 of the 68 adult subjects with positive genetic diagnosis, a disease penetrance of 65%. By comparison, an LV mass criterion of >180 gm identified HCM in only 14 of the 68 adult subjects, a disease penetrance of only 21%, $p<0.0001$.

4.3.2. Relation of IGF-I Levels to Age

Age-related IGF-1 concentrations were highly variable in the 150 subjects. However, IGF-1 concentrations, as reported previously, (Juul, 2001; Lannsen et al, 2001; Strasburger et al, 2001, Stratakis et al, 1996) increase with age reaching a maximum at 10 to 15 years, and then decline after 20 years of age. ***Figure 4-2.***

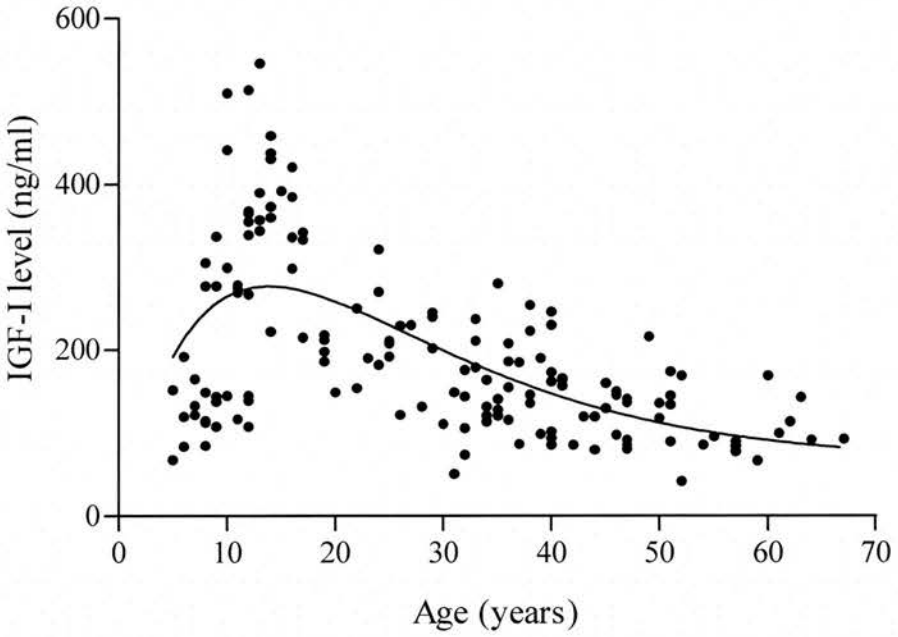

Figure 4-2: Relation of IGF-I levels to age

4.3.2. Clinical Findings

As IGF-I levels decline beyond the age of 20, the groups were further divided by age into adults and young patients and comparisons made between these groups. There were no significant differences between the groups with regards to age, gender, and body surface area. Young patients without sarcomeric mutation had slightly higher blood pressure compared to patients with sarcomeric mutations.

Table 4-2.

	Adults (≥20 years)			Young Patients (<20 years)		
	Group 1 n=44	Group 2 n=25	Controls n=23	Group 1 n=10	Group 2 n=21	Controls n=27
Age (years)	40±2	38±2	43±2	13±1	12±1	11±1
Gender (males)	24 (55%)	8 (32%)	8 (35%)	5 (50%)	6 (26%)	13 (48%)
BSA (m^2)	1.9±0.03	1.7±0.04	1.9±0.04	1.5±0.13	1.3±0.07	1.4±0.08
systolic BP (mm Hg)	122±3	116±2	125±4	104±3	106±3	114±2*
diastolic BP (mm Hg)	72±2	68±2	73±2	59±2	61±2	66±2**
Bonferroni multiple comparisons test; - *p<0.05 compared to group 1 and 2; **p<0.01 compared to group 1						

Table 4-2: Clinical characteristics of patients and controls in IGF-I study

Indices of cardiac hypertrophy by echocardiography and MRI were significantly greater in group 1 (Mutation +ve, phenotype +ve) compared to group 2 (mutation +ve, phenotype –ve) and controls (mutation –ve) in both adults and young patients. ***Table 4-3 and Table 4-4.*** In addition the QTc of adult patients was longer in group 1 compared to group 2 and controls. Young patients with sarcomeric mutations with and without LV hypertrophy had smaller LV internal dimensions by echocardiography compared to controls and young patients without sarcomeric mutations (controls) had greater peak systolic and diastolic blood pressure with exercise despite similar exercise tolerance.

	Adults (≥20 years)			
	Group 1	Group 2	Controls	ANOVA
12 lead ECG				
heart rate (bpm)	69±2	67±3	67±2	ns
PR interval (ms)	169±5	155±4	160±4	ns
QRS width (ms)	91±2	86±2	86±2	ns
QT interval (ms)	398±6	377±6	381±5	p<0.05
QTc	423±4	399±6*	400±5*	p<0.0005
Echocardiogram				
septum (mm)	20±0.9	10±0.4**	10±0.2**	p<0.0001
posterior wall (mm)	10±0.2	9±0.2	9±0.2	ns
LVIDd (mm)	44±1	45±1	44±1	ns
LVIDs (mm)	26±1	28±1	28±1	ns
left atrium (mm)	42±1	35±1**	34±1**	p<0.0001
FS (%)	42±1	38±2	41±1	ns
MRI				
$LVWT_{max}$ (mm)	24±0.9	13±0.6**	12±0.4**	p<0.0001
LV mass (g)	176±15	108±6**	112±6*	p<0.0001
EDV (ml)	111±6	101±4	111±5	ns
ESV (ml)	29±2	29±2	34±2	ns
EF (%)	74±1	72±2	69±1	ns
SV (ml)	82±5	73±4	76±4	ns
Ambulatory ECG				
heart rate (bpm)	70±1	75±3	72±3	ns
SVT	11±4	3±2	3±3	ns
non-sustained VT	7±2	0	1±1	ns
Exercise test				
duration (s)	549±29	503±35	620±42	ns
peak sBP (mm Hg)	153±3	149±5	163±4	ns
peak dBP (mm Hg)	75±2	78±4	79±3	ns
O^2 consumption (ml/kg/min)	19±1	22±2	22±2	ns
Bonferroni multiple comparisons test; *p<0.01, **p<0.0001 versus group 1; Group 1, mutation +ve, phenotype +ve; Group 2, mutation +ve, phenotype –ve; controls, mutation –ve.				

Table 4-3: Clinical findings of adult patients and controls in IGF-I study

	Young Patients (<20 years)			
	Group 1	Group 2	Controls	ANOVA
12 lead ECG				
heart rate (bpm)	74±4	77±3	78±4	ns
PR interval (ms)	142±7	140±3	138±3	ns
QRS width (ms)	95±7	80±2	81±2	ns
QT interval (ms)	382±10	359±7	362±6	ns
QTc	422±11	401±5	408±3	ns
Echocardiogram				
septum (mm)	16±2.5	8±0.7**	7±0.2**	p<0.0001
posterior wall (mm)	9±0.3	7±0.2**	7±0.2**	p<0.0001
LVIDd (mm)	40±3	38±1†	43±1	p<0.05
LVIDs (mm)	21±2††	22±1††	27±1	p<0.0001
left atrium (mm)	38±3	29±1**	29±1**	p<0.0001
FS (%)	45±2	41±1	38±1**	p<0.005
MRI				
$LVWT_{max}$ (mm)	19±1.3	11±0.4***	11±0.6***	p<0.0001
LV mass (g)	115±14	64±5**	73±6***	p<0.0002
EDV (ml)	99±9	76±5	85±5	ns
ESV (ml)	27±4‡	18±1	25±2‡	p<0.005
EF (%)	73±2	76±1	72±1	ns
SV (ml)	60±6	58±4	61±3	ns
Ambulatory ECG				
heart rate (bpm)	83±4	82±2	80±2	ns
SVT	0	0	0	ns
non-sustained VT	1±3	1±1	0	ns
Exercise test				
duration (s)	653±82	605±41	674±37	ns
peak sBP (mm Hg)	137±8	146±5	159±5*	p<0.05
peak dBP (mm Hg)	58±3	67±3	77±4**	p<0.005
O^2 consumption (ml/kg/min)	26±3	29±2	31±2	ns

Bonferroni multiple comparisons test; *p<0.05, **p<0.01, ***p<0.001 versus group 1; ‡p<0.05 compared to group 2; †p<0.05, ††p<0.0001 versus group 3; Group 1, mutation +ve, phenotype +ve; Group 2, mutation +ve, phenotype –ve; controls, mutation –ve.

Table 4-4: Clinical findings of young patients and controls in IGF-I study

Young subjects aged 5 to 19 years: The regression lines describing the relation between age and IGF-1 concentrations in groups 1 and 2 subjects, and normal controls (group 3) were similar. (The number of subjects was too small to test for differences in groups 1 and 2.) ***Figure 4-3.***

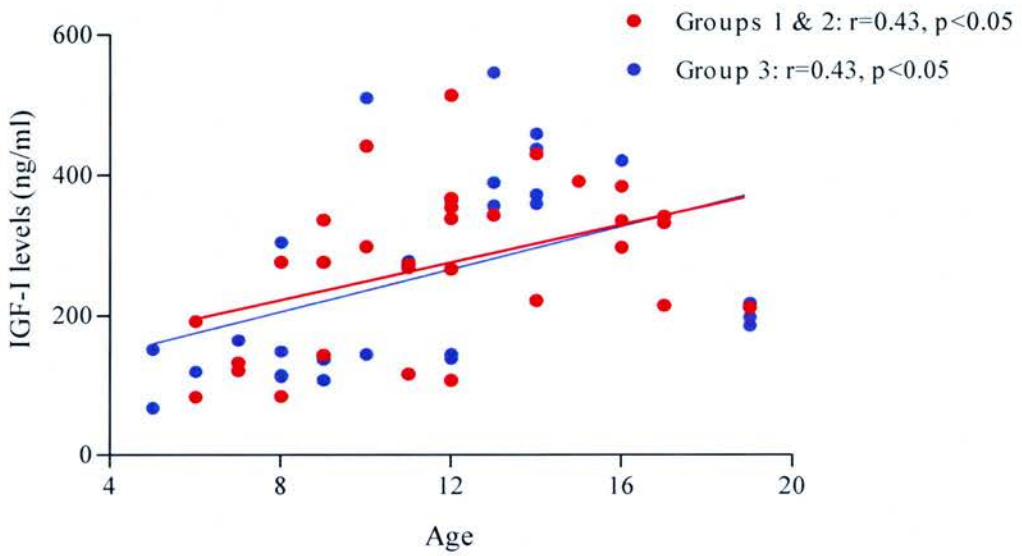

Figure 4-3: Relation of IGF-I levels to age in young patients

Adult subjects aged 20 to 67 years: There were no differences among the slopes of the regression lines describing the relation between age and IGF-1 in adults with HCM, adults with disease mutation without HCM and normal controls ($p=0.54$) but there were significant differences among the intercepts ($p<0.05$). This indicates that IGF-1 concentrations were lower in the subjects with LV hypertrophy (HCM). ***Figure 4-4.***

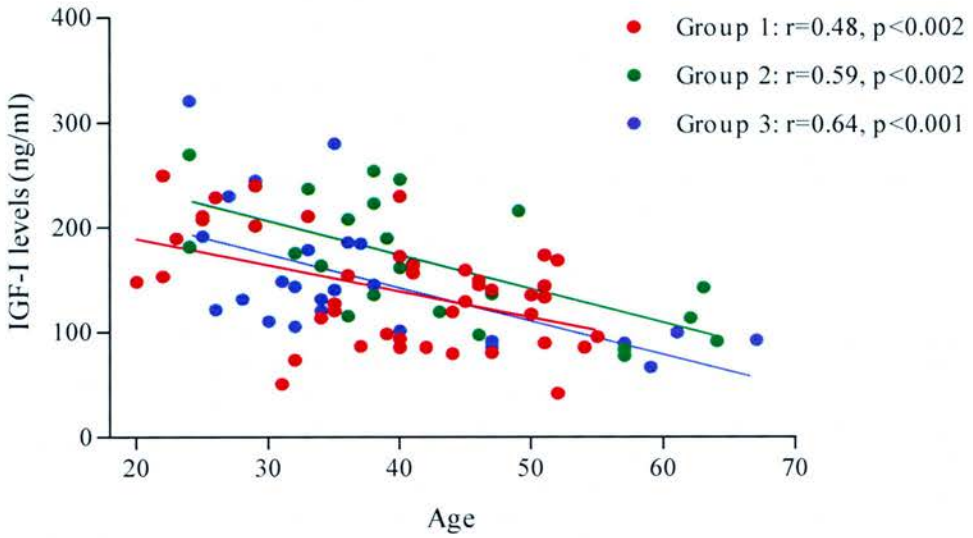

Figure 4-4: Relation of IGF-I levels to age in older patients

4.3.4. Relation of IGF-I Levels to Cardiac Indices in HCM

There was no correlation between IGFBP-3 concentrations and severity of cardiac hypertrophy or indices of cardiac function. However, there was a highly significant negative correlation between IGF-1 concentrations and maximum LVWT following correction for potentially confounding factors (age, gender, body surface area, IGFBP3, and family relationships). Notably, there was no correlation between IGF-1 concentrations and LV mass, presence or absence of LV outflow obstruction, or heart failure as demonstrated by level of exercise performance. ***Table 4-5.***

Variable	Mean	p^a	p^b
LV mass (g)	129±8	0.48	0.16
$LVWT_{max}$ (mm)	18±0.7	<0.02	<0.01
$LVWT_{max}$:EDV (mm/ml)	0.19±0.01	<0.0005	<0.0005
p^a After adjustment for familial relationship; p^b After adjustment for familial relationship, age, gender, BSA, BP, and IGFBP3			

Table 4-5: Relation of IGF-I levels to cardiac indices in HCM

IGF-1 concentrations and LV function: LV wall stress is inversely related to LVWT, and directly to LV volume. Notably, in the present study $LVWT_{max}$:EDV ratio was independent of age and was highly significant and independent correlate of IGF-1 concentrations.

HCM subjects tended to have higher LV ejection fractions and circumferential shortening. However, there was no direct correlation between IGF-1 concentrations and LV systolic function.

4.4. Discussion

Cardiac hypertrophy is a major independent risk factor for morbidity and mortality from cardiovascular disease. (Levy *et al.* 1990) Severe LV hypertrophy in HCM has also been reported to be associated with a poor prognosis. (Spirito *et al.* 2000) In this study of the role of circulating IGF-1 in determining magnitude of LV hypertrophy in HCM, we found that the plasma concentrations of the hormone were lower in HCM patients and correlated negatively with $LVWT_{max}$. There was, however, no correlation between its binding protein, IGFBP3 concentrations, and cardiac hypertrophy. These findings are at variance with previous reports.

These conclusions depended on certain experimental considerations that apply critically to all evaluations of potential modifiers of phenotypic expression in HCM. First, we required a sensitive method to quantify and compare cardiac structure and function. Previously, echocardiography has been used to estimate LV mass and volumes. However, the calculations rely on assumptions of LV morphology that may not be valid as the LV in HCM is often distorted and the distribution of the LV hypertrophy varies markedly. (Wigle *et al.* 1985) Notably, the

hypertrophy affects primarily the anterior inter-ventricular septum in the common asymmetrical septal hypertrophy variety. The hypertrophy affects the distal half of the LV in apical HCM, and prominently the LV at the level of the papillary muscles in mid-cavity HCM. In contrast, only a relatively small part of the LV is thickened and the rest of the heart is relatively normal in focal HCM. MRI estimates of LV mass, $LVWT_{max}$ and LV volumes are based on direct examination of multiple slices of the LV from base to apex with clear presentations of regional LV wall thickness. Second, the relatively large number of subjects with a genetic diagnosis, and the diversity of families and mutations causal for HCM, reduced the bias due to undue influence of a certain mutation or inherited modifying factor on phenotypic expression. We further corrected for the position of members in families as family relationships may also affect inheritance of modifying factors. Third, as the concentrations of hormones such as IGF-1 differ in the young compared to adult patients, we analysed the data separately for the two age groups. Finally, we corrected for other potentially confounding factors such as body surface area, gender, and concentrations of the carrier protein IGFBP3.

Our initial hypothesis was that given the trophic actions of the hormone (Fazio *et al.* 2000), high IGF-1 concentrations would correlate with more severe cardiac hypertrophy in HCM, but unexpectedly, found the converse to be the case. The explanation for this finding may lie in the proposed aetiology of the cardiac hypertrophy in HCM, and the ability of IGF-1 to improve myocyte function.

Genetic causes of HCM consist mostly of single substitutions of a conserved amino acid that are incorporated into the sarcomere where they presumably act as 'poison peptides'. Alternatively, in some instances HCM is associated with deletion

mutations, and hence, presumably the disease may also be caused by haplo-insufficiency. The primary abnormality therefore in both cases is malfunctioning sarcomeres and the LV hypertrophy is compensatory and mediated by 'modifying' factors. This view is supported by several observations:

- in HCM caused by missense mutations of several genes result in abnormal function of the acto-myosin molecular motor (Cuda *et al.* 1997; Palmiter *et al.* 2000)
- single skinned slow skeletal myofiber expressing mutant cardiac myosin alter isometric force, power, and unloaded shortening velocity (Lankford *et al.* 1995)
- tagging MRI studies have shown impaired regional LV wall contraction in HCM (Kramer *et al.* 1994)
- highly variable phenotypic expression in family members with the identical disease mutation (Fananapazir and Epstein 1994)
- the cardiac hypertrophy often develops and progresses during puberty with rapid body growth (Maron *et al.* 1986a)
- cardiac hypertrophy involves proliferation of several cell types that do not express the mutant gene. (Ferrans 1998)

As the hypertrophy and impairment of contraction are not uniform throughout the ventricle, regional differences in LV contractility may act as a stimulus for maintenance of the LV hypertrophy. Increased cytosolic Ca^{2+} and sensitivity of the acto-myosin molecular motor to Ca^{2+}, may further aggravate sarcomeric dysfunction. (Karibe *et al.* 2001; Marian 2000) Abnormal myocyte stretch and stress may be the

stimulus for local release of hormones that, in addition to circulating hormones, are responsible for hypertrophy and proliferation of several cell types, characteristic of the compensatory LV hypertrophy. Myocardial ischemia caused by excessive oxygen demand and abnormal intra-myocardial blood vessels may contribute to the cardiac remodelling. (Fananapazir 1999)

Thus, in contrast to conditions associated with increased afterload, such as hypertension, normal systemic pressures in HCM are perceived as an excessive load by a myocardium with impaired contractile properties. This leads to similar compensatory LV hypertrophy through perhaps the action of myocardial trophic factors or circulating growth factors. The cardiac hypertrophy is, however, maladaptive. It reduces LV wall tension and augments systolic function in the short and medium term, but in the long term, myocyte necrosis, fibrosis, and myocellular energy depletion, lead to cardiac failure.

Our findings therefore lead us to postulate that high concentrations of circulating IGF-1 improve failing myocytes, by helping to compensate for sarcomeric dysfunction and reduce the stimulus for development of the maladaptive cardiac hypertrophy by improving muscle efficiency. A notable observation was that the IGF-1 concentrations correlated better with maximum LV wall thickness than LV mass. This may be explained by the fact that expression of mutant contractile protein and IGF-1 in the myocardium, are not uniform, and further, the LV walls are subject to different stresses and strains. (Cuda *et al.* 1997; Li *et al.* 2002)

Our findings are at variance with reports of a direct association between IGF-1 and IGFBP3 concentrations and LV hypertrophy in HCM (Demirtas *et al.* 1998;

Saeki *et al.* 2002), and provide evidence that the use of antagonists of GH and IGF-1 in HCM may have deleterious long-term results.

As IGF-1 prevents myocyte apoptosis, the role of declining IGF-1 concentrations with advancing age in the pathogenesis of heart failure caused by myocyte death and replacement by fibrous tissue observed in some patients requires further evaluation.

4.4.1. Limitations

We did not quantify IGF-1 receptors or autocrine/paracrine activities of IGF-1 by measuring myocardial tissue concentrations of the hormone. These data are, however, difficult to obtain or to interpret as IGF-1 concentrations may well vary significantly in different regions of the myocardium.

4.5. Conclusions

HCM is appears to be less severe when associated with high physiological concentrations of circulating IGF-1. Identification of this important modifier of phenotypic expression will improve our risk stratification and permit development of novel therapeutic strategies to treat the HCM.

5. Brain Natriuretic Peptide and Disease Severity in HCM

5.1. Introduction

Brain natriuretic peptide was originally isolated from porcine brain, (Sudoh *et al.* 1988) hence the name and subsequently from porcine heart. (Hasegawa *et al.* 1991; Saito *et al.* 1989) It shares considerable homology with atrial natriuretic peptide (ANP) but there are significant differences. BNP is synthesized as a large molecular weight pro-hormone (N-terminal pro-BNP), however, there is little storage of pro-BNP and therefore any augmentation of production of BNP will require a prolonged stimulus. Although, like ANP, BNP is synthesized in atrial tissue, it is predominantly produced in ventricular tissue. (Mukoyama *et al.* 1991; Yasue *et al.* 1994) Post-translational processing of pro-BNP occurs in cardiomyocytes; however, both high molecular weight pro-BNP and BNP appear in the circulation. (Yandle *et al.* 1993) Most radio-immunoassays of BNP actually measure both forms, however as there is such a strong correlation between these two levels, (Yandle *et al.* 1993) the total level is still a good measure of augmented production.

BNP levels are elevated in a number of pathological states involving either cardiovascular or renal disease. ***Table 5-1*** The mechanisms by which these diseases cause augmentation of BNP levels include:

- intravascular volume overload
- increased central venous pressure
- tachycardia
- impaired renal function.

Cardiovascular conditions causing elevated levels of BNP are summarised in the table below. (Sagnella 1998)

Disease	BNP	Reference
Essential hypertension	↑	(Buckley *et al.* 1993)
Tachycardias	↑	(Kohno *et al.* 1992)
Heart failure	↑↑↑	(Yandle *et al.* 1993)
Isolated diastolic dysfunction	↑↑	(Lang *et al.* 1994)
Mitral stenosis	↑↑	(Matsumoto *et al.* 1995)
Aortic stenosis	↑↑↑	(Ikeda *et al.* 1997)
Dilated cardiomyopathy	↑↑↑↑	(Matsumoto *et al.* 1995)
Hypertrophic cardiomyopathy		
non-obstructive	↑↑↑	(Hasegawa *et al.* 1993)
obstructive	↑↑↑↑	(Hasegawa *et al.* 1993)
Myocardial infarction		
on admission	↑↑↑	(Morita *et al.* 1993)
during recovery	↑↑↑↑	(Morita *et al.* 1993)
Chronic renal failure		
dialysis-independent	↑↑	(Buckley *et al.* 1992)
dialysis-dependent	↑↑↑	(Lang *et al.* 1992)
Approximate relative increase compared to controls: ↑, up to 3 fold; ↑↑, ~3-10 fold; ↑↑↑, ~ 10-30 fold; ↑↑↑↑, >30 fold		

Table 5-1: Relative increase in BNP in different pathological states

BNP causes vasodilatation and natriuresis and counteracts the effects of the adrenergic, renin-angiotensin-aldosterone and anti-diuretic hormone systems and therefore, have a beneficial effect in heart failure. (Saito *et al.* 1987; Yoshimura *et al.* 1991) Infusion of BNP decreases arterial and venous pressure, increase cardiac output and suppresses neuro-hormonal activation.

BNP levels are elevated in patients with HCM and this increased level of BNP is further enhanced by the presence of LV outflow tract obstruction. (Hasegawa *et al.* 1993) In patients with HCM, BNP levels have also been shown to be independently associated with NYHA functional class and $LVWT_{max}$. Unfortunately, there is considerable overlap between levels and functional class, with significantly elevated levels occurring in patients who were otherwise asymptomatic or minimally symptomatic, limiting its clinical utility. (Maron *et al.* 2004)

Although there have been studies exploring the prognostic significance of BNP levels in heart failure this has not been applied to patients with HCM. In this study we investigated the relationship between BNP levels and hypertrophic cardiomyopathy in group of patients with defined mutational status.

5.2. Methods

Thirty-seven subjects, in whom the disease causing mutation was known, participated in this study allowing inclusion of subjects without significant LV hypertrophy despite a disease causing mutation. Individuals with LV outflow tract obstruction were excluded from the study because of the affect of augmented LV pressure on BNP levels. An additional 19 related subjects without HCM mutation were included as controls. The 56 patients and controls were recruited sequentially from subjects participating in the previous study. All patients were in sinus rhythm.

All subjects underwent mutational analysis, clinical examination, ergometric upright bicycle exercise testing with breath-by-breath gas analysis and MRI. All studies were performed at least 48 hours off all medications.

Blood for plasma BNP estimation was withdrawn after at least 30 minutes rest and measured by Cardiorenal Lab, Mayo Clinic using previously published methods.

5.2.1. Mutational Subgroups

Seven sarcomeric gene mutations (7 families) were identified in the 37 HCM subjects. These mutations were absent in 19 normal family members included as controls. Group 1 consisted of 22 of the 37 patients in whom the sarcomeric mutation was associated with a benign prognosis. They belonged to 4 relatively large families in which only 2 premature deaths (< 55 years of age) have occurred in 116 (1.7%) affected family members. In contrast, group 2, consisted of 15 patients in whom the sarcomeric mutations have been associated with a high incidence of SCD. Twenty-five SCDs have occurred in 55 (45%) affected family members. (Fisher's exact test, $p<0.0001$) ***Table 5-2.***

Gene	Locus	Mutation	No. of Subjects	Sudden death/ Affected Subjects	No. of Controls
Group 1					
MYH7	14q3	$Asn^{187}Lys$	5	0/8 (0%)	0
		$Lys^{847}Glu$	6	0/51(0%)	1
		$Leu^{908}Val$	8	2/46 (4%)	10
ACTC	15q11-q14	$Glu^{99}Lys$	3	0/11 (0%)	0
Group 2					
MYH7	14q3	$Glu^{389}Gln$	5	8/14 (57%)	0
		$Arg^{403}Gln$	2	6/15 (40%)	1
TPM1	15q22	$Val^{95}Ala$	8	11/26 (42%)	7

Table 5-2: Mutational subgroups of patients included in BNP study

5.2.2. Statistics

Data are expressed as mean ± SEM. Two-sample continuous variables were compared using Student's t-test. Multiple groups were compared using one-way analysis of variance and differences between individual groups were compared using Bonferroni multiple comparisons test. Spearman's correlation was used to test association between two continuous variables and analysis of co-variance was performed to examine the relation of BNP to other variables. A p-value <0.05 was considered significant.

5.3. Results

The two groups and controls did not differ significantly with regards to age, gender, BSA, systolic blood pressure, or functional class, although the diastolic BP of group 1 was significantly higher than group 2. ***Table 5-3.***

	Group 1: Benign Mutations n=22	Group 2: Malignant Mutations n=15	Controls n=19
Age (years)	28±3	25±3	25±4
Gender (males)	9 (41%)	7 (47%)	5 (26%)
BSA (m^2)	1.69±0.07	1.68±0.10	1.58±0.10
systolic BP (mm Hg)	113±3	111±3	112±3
diastolic BP (mm Hg)	71±3*	63±2	65±2
NYHA functional class	1.3±0.1	1.4±0.2	N/A
Bonferroni multiple comparisons test;- *p<0.01 compared to group 2			

Table 5-3: Clinical characteristics of patients and controls in BNP study

HCM patients had significantly greater LV mass, $LVWT_{max}$, higher EF and larger left atrium (LA). When indexed to body surface area, HCM patients had

greater $LVWT_{max}$-I and LV mass-I. LA-I of patients in group 2 was greater than that of patients in group 1 and controls. There were no differences between the groups with regards to exercise duration or MVO_2. However, group 2 had a significantly lower peak systolic BP compared to controls ($p<0.05$).

	All HCM	Group 1	Group 2	Controls
BNP level (ρg/ml)	131±37*	50±18	251±80*†	28±5
MRI				
$LVWT_{max}$ (mm)	18±1***	17±2*	19±1**	12±1
LV mass (g)	114±9*	112±13	119±13	85±8
LA (mm)	51±1***	47±1*	56±2**††	41±1
EDV (ml)	100±4	100±6	99±7	96±8
ESV (ml)	27±2	25±2	29±3	29±3
EF (%)	74±1*	76±1*	71±2	70±1
SV (ml)	73±3	75±5	70±5	67±6
MRI (Indexed to BSA)				
$LVWT_{max}$-I (mm/m^2)	10.7±0.5***	10.0±0.7*	11.7±0.8**	7.8±0.3
LV mass-I (g/m^2)	66±4*	64±5	69±5	52±3
LA-I (mm/m^2)	31±1	28±1	36±3*†	27±2
EDV-I (ml/m^2)	59±2	59±3	59±2	61±2
ESV-I (ml/m^2)	15±1*	14±1*	16±1	18±1
SV-I (ml/m^2)	44±2	45±2	43±2	42±2
Exercise test				
duration (s)	427±31	446±39	403±52	361±39
peak sBP (mm Hg)	146±4	154±4	135±6*	160±6
peak dBP (mm Hg)	74±3	75±4	72±4	74±3
O^2 consumption (ml/kg/min)	25±2	26±2	24±5	26±2

Bonferroni multiple comparisons test;- *p<0.05, **p<0.005, ***p<0.0005 compared to controls; †p<0.05, ††p<0.001 compared to group 1; Group 1, benign mutations; Group 2, malignant mutations

Table 5-4: Clinical findings in patients and controls in BNP study

Plasma BNP levels varied markedly but were on average 5 times higher in the HCM patients compared to controls (131±37 ρg/ml versus 28±5 ρg/ml, $p<0.01$). In addition, plasma BNP levels were significantly greater in group 2 (malignant mutations), compared to both group 1 (benign mutations) and controls. ***Figure 5-1.***

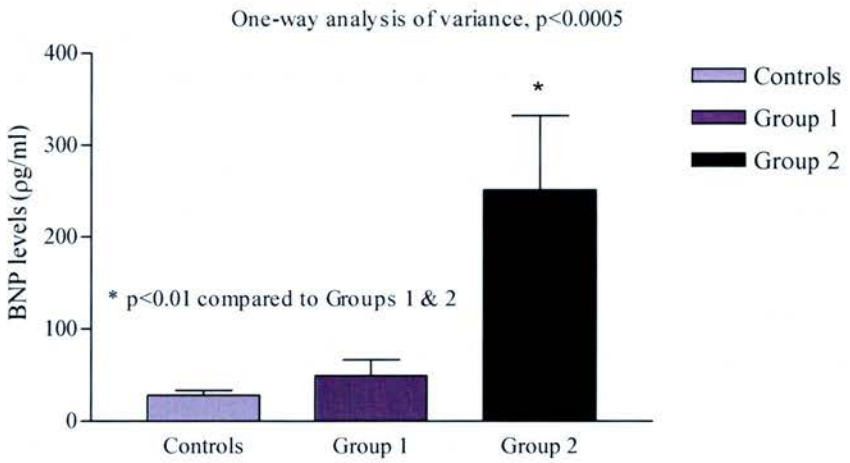

Figure 5-1: BNP levels in the three groups

There was no correlation between cardiac indices by MRI and BNP. However, after indexing to BSA, LA-I, LV mass-I and $LVWT_{max}$-I significantly correlated with BNP. ***Figure 5-2.*** There was no correlation between BNP, and age, gender, blood pressure, cardiac index, ejection fraction, and LV volumes indexed to BSA.

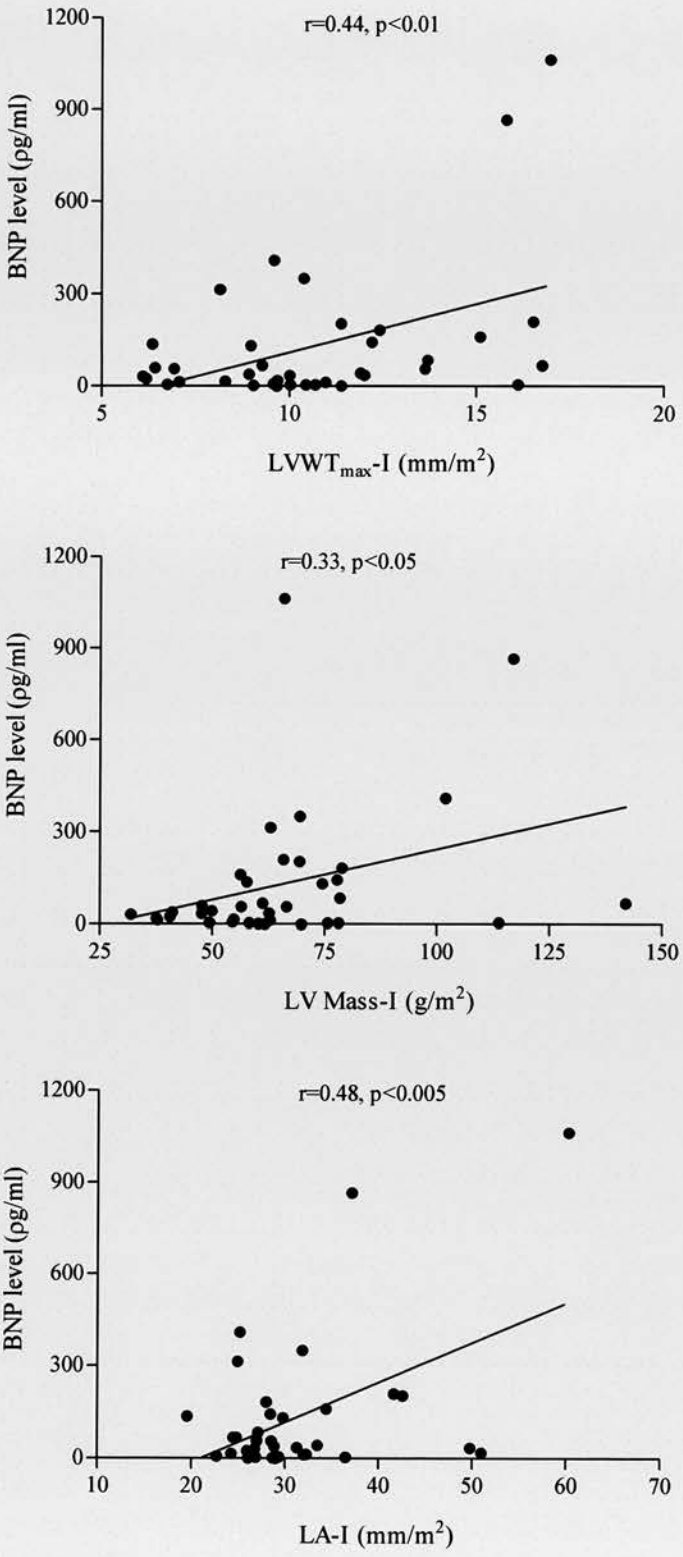

Figure 5-2: Scatter plots of correlation between BNP and cardiac indices

As the distribution of BNP levels in HCM patients was positively skewed (3.02±0.39), the data was transformed by taking the log normal (Ln) value of BNP which normalised the distribution (-0.24±0.39). Stepwise regression including BNP (dependent variable), age, gender, mutational group, blood pressure, LA-I, LVM-I, and LVWT-I demonstrated mutational group as the only independent predictor of Ln BNP levels ($p<0.05$).

5.4. Discussion

HCM is characterized by marked genetic and phenotypic heterogeneity. Although several prognostic factors have been identified, including the nature of the molecular defect causing the disease, (Epstein *et al.* 1992; Marian *et al.* 1995; Yamauchi-Takihara *et al.* 1996) risk evaluation of affected patients remains difficult. Some patients with a sarcomeric mutation, but with mild or no LV hypertrophy may still be at risk for sudden death which further complicates the problem of risk stratification. (Karibe *et al.* 2001; Varnava *et al.* 2001; Watkins *et al.* 1995) In addition, knowledge of why certain sarcomeric mutations are associated with a higher incidence of sudden death and the exact mechanism of sudden death is limited.

BNP is related to atrial natriuretic peptide but is secreted predominantly by LV myocytes. (Yasue *et al.* 1994) Human BNP levels are increased in conditions associated with volume over- and after-load, LV hypertrophy, and LV systolic and diastolic dysfunction, such as hypertension, mitral regurgitation, aortic stenosis and regurgitation, dilated cardiomyopathy, and myocardial ischemia. HCM is associated with LV hypertrophy, myocardial ischemia and diastolic dysfunction. In keeping

with this, Hasegawa *et al*, have reported that myocardial tissue expression of BNP is several times higher in patients with HCM, particularly those with LV outflow tract obstruction.

We report on the clinical associations of plasma BNP levels in patients in whom the molecular cause of HCM has been determined, and who had detailed MRI evaluation of LA and LV dimensions. No patient had outflow tract obstruction or depressed LV function. Knowing the genetic status also allowed participation of family members with the same genetic background as the HCM patients in whom the disease mutation was known to be absent who could act as controls.

Utilization of MRI to quantify LV structure and function also provided distinct advantages. The LV is significantly distorted in HCM and hence, LV mass derived from echocardiographic indices is likely to be inaccurate due to several assumptions that are included in the calculation. In addition, the site of maximum LV wall thickness may not be the proximal inter-ventricular septum but in an area that is difficult to assess through standard echocardiographic windows. As would be expected, LV mass and LV wall thickness indices varied markedly in our patients, even in those with identical mutation.

Patients with mutations associated with a high incidence of sudden death had significantly higher BNP levels than those patients with mutation associated with a benign prognosis despite similar LVM-I, LVWT-I and symptoms. However, LA-I was significantly greater in those patients with malignant mutations compared to both those with benign mutations and controls suggesting that these individuals may be more likely to have had factors associated with elevated BNP levels such as systolic or diastolic dysfunction. However, 3 asymptomatic individuals in this group

with minimal or no LV hypertrophy and normal systolic function had markedly elevated plasma BNP levels. Therefore, mutations associated with a poor outcome may have effects on myocardial properties (myocardial ischemia, diastolic function etc), even in the absence of appreciable LV hypertrophy.

5.4.1. Limitations

Rest and exercise haemodynamic findings were not available in our patients. The HCM subjects with a malignant mutation were more likely to have abnormal blood pressure responses to exercise. Hence, we cannot be certain that diastolic dysfunction and elevated filling pressures are not also important determinants of BNP levels. This is more likely given that there was a significant difference in LA dimension. However, only a signal linear measure of LA dimension was available. It is clear, however, that patients with malignant mutations may have elevated BNP levels despite absence of congestive symptoms or LV hypertrophy. It may be that exercise BNP levels correlate better than resting BNP values with clinical findings and prognosis in HCM, particularly, as myocardial ischemia detected by exercise thallium scintigraphy is present in about two-thirds of HCM patients and may be an important cause of sudden death in patients in whom HCM is caused by malignant mutations. A further important study may be to relate resting and exercise BNP levels prospectively to subsequent cardiac events.

5.5. Conclusions

Plasma BNP levels are markedly elevated in patients with HCM, and this is in part determined by the sarcomeric mutation responsible for the disease

independent of the degree of LV hypertrophy present. Plasma BNP levels vary markedly in our patients with HCM, ranging from 1.6 - 1062 pg ml^{-1}, limiting its utility as a marker of severity of disease. However elevated plasma BNP levels in the absence of significant LV hypertrophy may indicate abnormalities of LV function that could influence outcome. Members of families with a malignant history of HCM and no LV hypertrophy should be considered at risk of sudden death if a disease causing mutation cannot be excluded.

6. Genetic Modifiers of Phenotypic Expression in HCM

6.1. Introduction

In contrast to genetic mutations, polymorphisms are variations in individual DNA sequences found in individuals, groups or populations that may give rise to certain characteristics. The least frequent allele or genetic marker occurs more frequently that can be accounted for by mutation alone. These polymorphisms may either alter the expression or function of the final product without in isolation causing disease. However, as a result of their modification of the final product, they may influence the course of other disease processes. A number of genetic polymorphisms have been implicated in the modification of a variety of cardiovascular diseases including coronary artery disease (Nakai *et al.* 1994) and HCM. (Brugada *et al.* 1997; Marian *et al.* 1993; Osterop *et al.* 1998)

Angiotensin-I converting enzyme is a zinc metallo-peptidase whose main function is to convert angiotensin-I (AG-I) into angiotensin-II (AG-II) and inactivate bradykinin. AG-II is a potent vasoconstrictor, stimulates the production of aldosterone and may stimulate the development of cardiac hypertrophy. There is wide inter-patient variation in ACE levels but they remain fairly consistent within individuals. Familial aggregation of ACE levels suggested that its production is under genetic control. A polymorphism in the ACE gene influences its expression. (Rigat *et al.* 1990) The polymorphism consists of the presence or absence of a 250 base pair (bp) insertion in a non-coding section (intron 16) of the gene. Subjects homozygous for the deletion (DD) have higher levels of ACE than heterozygous

subjects (ID) and subjects homozygous for the insertion (II). The DD genotype has been associated with hypertension and the development of LV hypertrophy in cardiomyopathy (Marian *et al.* 1993) and aortic stenosis. (Wong *et al.* 1996) It has also been related to the risk of SCD in HCM. (Marian *et al.* 1993)

Endothelin-1 (ET-1) is a 21 amino acid peptide and is a potent vasoconstrictor produced by endothelial and smooth muscle cells. In addition to being vasoactive, it has been implicated in mediating the hypertrophic response of a number of cell types and stimulates the production of other hormones. (Ito *et al.* 1991; Shubeita *et al.* 1990) It was originally isolated from porcine endothelial cells and has since been cloned and sequenced. A G/A polymorphism at position 8002 in intron 4 of the ET-1 gene has previously been identified. Although it is unlikely to directly influence the expression or function of ET-1 it may be in linkage disequilibrium with other functional polymorphisms that could influence the development of LV hypertrophy in HCM.

The ACE gene polymorphism has previously been shown to influence the development of LV hypertrophy in patients with HCM. The ET-1 gene polymorphism has also been demonstrated to influence LV hypertrophy, in conjunction with gender, in HCM (Brugada *et al.* 1997) and may influence the propensity to malignant cardiac arrhythmias in patients with coronary artery disease. (Kozak *et al.* 2002)

In this study we re-examined the relationship between these two polymorphisms in a larger cohort of patients with HCM.

6.2. Methods

This study was performed after informed consent in 229 patients with HCM in whom DNA had been extracted for analysis. The genetic cause of HCM was known in 109/229 (48%) patients which allowed inclusion of family members with an HCM causing mutation but with little or no LV hypertrophy. All patients underwent screening echocardiography and cardiac MRI. Indices of cardiac hypertrophy were correlated with ACE and ET-1 genotype.

6.2.1. Genetic Analysis

DNA from patients with HCM was extracted from whole blood using standard techniques and stored in 100ng/μL concentrations. This was subsequently diluted to 20ng/μL concentrations for further analysis.

ACE gene polymorphism: Polymerase chain reaction (PCR) was used to detect the insertion (I)/deletion (D) polymorphism of the ACE gene. 5μL of 20ng/μL DNA, 1μL of 12.5ng/μL sense primer (5'CTGGAGACCACTCCCATCCTTCT3'), 1μL of 12.5ng/μL antisense primer (5'GATGTGGCCATCACATTCGTCAGAT3') and 18μL of water was added to Ready-To-Go RT-PCR beads (Amersham Pharmacia Biotech). The total reaction of 25μL was run on a GeneAmp PCR System 9700 for 30 cycles of 94°C for 1 min denaturation, 58°C for 1 min annealing, and 72°C for 1 min extension. The PCR product was run on an agarose gel with ethidium bromide staining. ***Figure 6-1.***

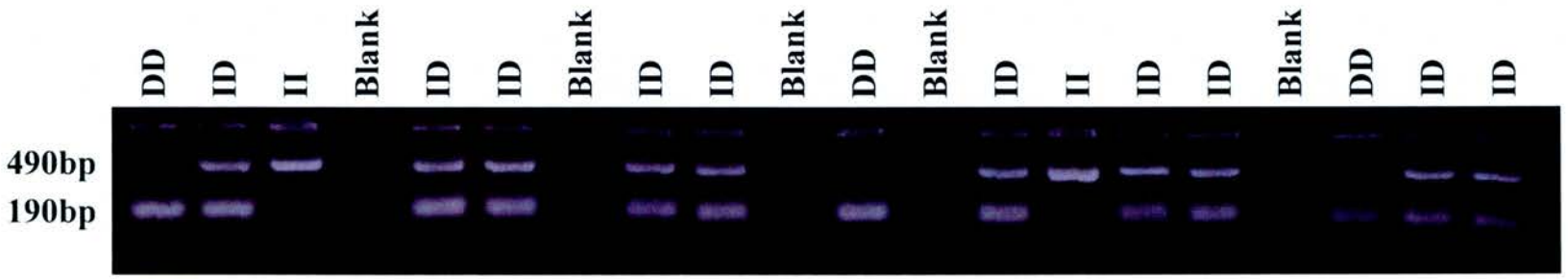

Figure 6-1: Ethidium bromide gel showing three patterns of PCR product demonstrating three ACE genotypes (490 bp insertion homozygote 'II', 190 bp deletion homozygote 'DD', and 490 bp/190 bp insertion/deletion heterozygote 'ID'). Blank channels indicate failed PCR and samples were re-run.

Endothelin gene polymorphism: A PCR was also performed on DNA to detect the ET-1 gene polymorphism. 5μL of 20ng/μL DNA, 1μL of 12.5ng/μL sense primer (5'AGTAGCAGAGAGATCTATGCATCC3'), 1μL of 12.5ng/μL antisense primer (5'CAGCATGTTCTAAATTCTACCAACCC3') and 18μL of water was added to Ready-To-Go RT-PCR beads (Amersham Pharmacia Biotech). The total reaction of 25μL run on a GeneAmp PCR System 9700 for 30 cycles of 94°C for 1 min denaturation, 65°C for 1 min annealing, and 72°C for 40 sec extension. The G allele has a Taq1 digestion site which results in 2 products of 230 bp and 144 bp. The presence of the G8002A polymorphism removes this digestion site and results in a single product of 374 bp. Therefore the PCR product was digested with Taq1 and run on agarose gel with ethidium bromide staining. To digest 15μL of PCR product was added to 1μL of the TaqI, 1μL of NE Taq buffer, 1μL of BSA and 32μL of water. The total reaction of 50μL was run at 65°C for 4 hours. (Brugada *et al.* 1997)

Figure 6-2.

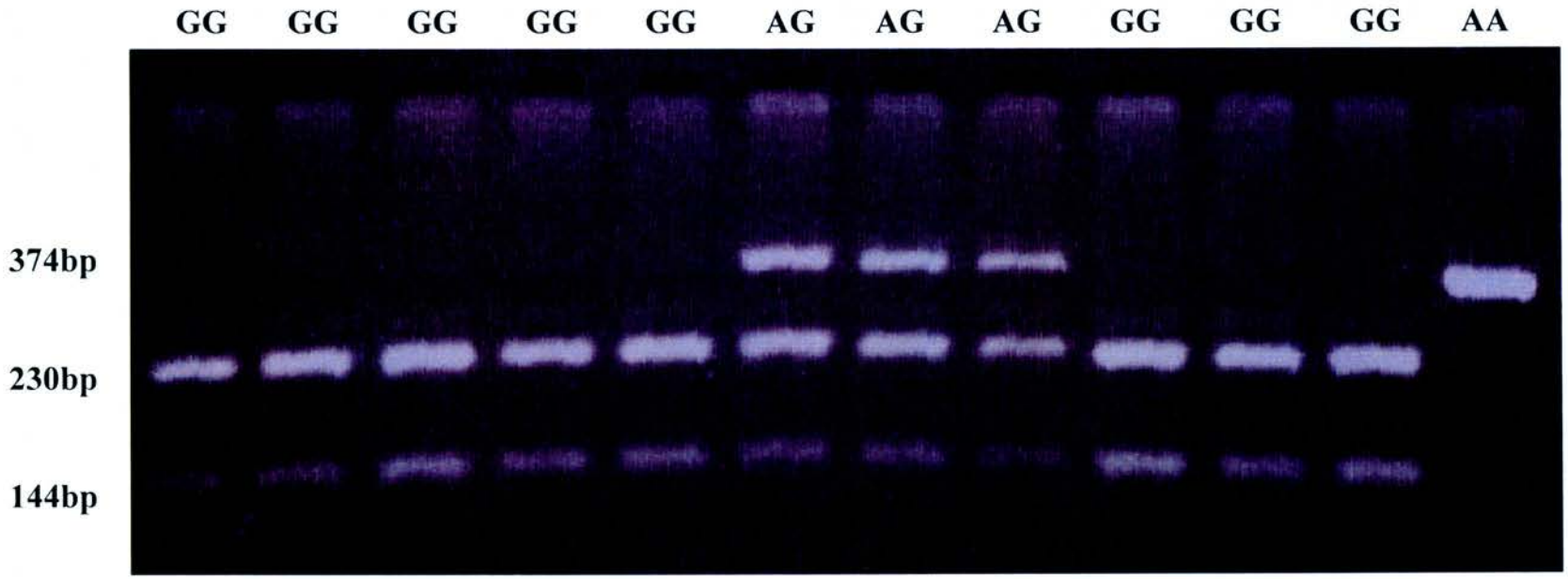

Figure 6-2: Ethidium bromide gel showing three patterns of PCR product following Taq1 digest demonstrating the G8002A ET-1 polymorphism. Homozygotes for the G allele had 2 products of 144 bp and 230 bp, homozygotes for the A allele had a single product of 374 bp and heterozygotes had all 3 products of 144 bp, 230 bp and 374bp.

6.2.2. Statistics

Results are expressed as mean ± SEM. Differences between groups were analysed using a one-way analysis of variance. To evaluate the relationship between ACE genotypes (II, ID, and DD) and left ventricular wall thickness, we used covariance techniques, which account for the familial relationships among the members of the study. A measured genotype approach of variance component analysis (Hopper and Mathews 1982) being implemented in the genetic software, SOLAR (Almasy and Blangero 1998) was used for the data analysis. This approach is able to deal with unrelated individuals as well as pedigrees of different sizes and structures. It fully accounts for the different types of relationship within a pedigree in performing association studies on the measured genotypes and quantitative traits. To eliminate the effect of potential confounders, for the analyses we adjusted for age, sex, and blood pressure.

6.3. Results

The 229 patients came from 148 families. Of the 109 patients with a known genetic defect, there were 28 mutations in 6 genes. ***Table 6-1.***

Gene	Locus	No. of Mutations	No. of Families	No. of Patients
MYH7	14q3	19	24	79
TPM1	15q22	1	1	12
ACTC	15q11-q14	3	3	11
MYL2	12q23	2	2	2
MYL3	3p21	1	1	1
MYBP3	11p11	2	2	4
Total		28	33	109

Table 6-1: Families and mutations of patients in genetic modifier study

6.3.1. ACE Gene Polymorphism

There were 43 patients homozygous for the insertion allele, 129 who were heterozygous and 57 homozygous for the deletion allele. There was no difference between the three groups with regards to age, gender, blood pressure or BSA.

	II n=43	ID n=129	DD n=57
Age (years)	36±3	36±1	35±2
Gender (males)	22 (51%)	68 (53%)	27 (47%)
BSA (m^2)	1.78±0.05	1.81±0.03	1.89±0.04
Systolic BP (mm Hg)	119±2	119±1	119±2
diastolic BP (mm Hg)	70±2	70±1	70±2

Table 6-2: Clinical characteristics of patients by ACE genotype

On echocardiography there was a gradual increase in LA size between (II) patients and (DD) patients. The same was true of septal thickness. There was no significant difference between the groups with regards to LV internal dimension, posterior wall thickness, or outflow tract obstruction. **Table 6-3.**

	II	ID	DD	ANOVA
Septum (mm)	16±1	18±1	20±1*	$p<0.05$
Posterior wall (mm)	9±0.2	10±0.2	10±0.3	ns
LVIDd (mm)	43±1	43±1	44±1	ns
LVIDs (mm)	26±1	26±1	25±1	ns
LA (mm)	37±1	39±1	42±1**	$p<0.01$
OT gradient (mm Hg)	11±4	14±2	10±3	ns
OT, outflow tract; Bonferroni multiple comparisons test, *$p<0.05$, **$p<0.01$ compared to II				

Table 6-3: Echo findings of patients by ACE genotype

MRI indices of cardiac hypertrophy were also compared. There was no difference in LV volumes between patients. Although patients who were either heterozygous or homozygous for the D allele had lower EF and greater LV mass, neither reached statistical significance. However, $LVWT_{max}$ was significantly greater in patients with the D allele. After indexing the cardiac MRI values to BSA, patients with the D allele had greater $LVWT_{max}$-I compared to patients homozygous for the I allele. ***Table 6-4.***

	II	ID	DD	ANOVA
$LVWT_{max}$ (mm)	19±1	22±1*	23±1**	p<0.01
LV mass (g)	167±16	189±9	198±12	ns
EDV (ml)	109±6	104±3	108±4	ns
ESV (ml)	27±3	27±1	28±2	ns
EF (%)	73±2	70±1	70±1	ns
SV (ml)	81±5	78±2	80±3	ns
$LVWT_{max}$-I (mm/m^2)	10.4±0.6	12.3±0.3*	12.3±0.4*	p<0.02
LV mass-I (g/m^2)	89±6	102±4	104±6	ns
EDV-I (ml/m^2)	60±2	58±1	57±2	ns
ESV-I (ml/m^2)	15±1	15±1	15±1	ns
SV (ml/m^2)	45±2	43±1	42±1	ns
Tukey-Kramer multiple comparisons test, *p<0.05, **p<0.01 compared to II				

Table 6-4: Cardiac MRI findings of patients by ACE genotype

Multivariable analysis using a measured genotype approach to account for the familial relationships was performed with age, gender and blood pressure included as possible confounders. $LVWT_{max}$-I was found to be independently significantly associated with ACE genotype.

6.3.2. Endothelin-1 Gene Polymorphism

There were 138 patients homozygous for the G allele, 84 patients heterozygous and 7 patients homozygous for the A allele. There were no differences in basic clinical characteristics, echocardiographic findings or cardiac MRI parameters between the different genotypes. ***Table 6-5.***

There was no association between ET-1 genotype and any variable by multivariable analysis.

	GG n=138	GA n=84	AA n=7
Age (years)	35±1	38±2	34±5
Gender (males)	67 (49%)	46 (55%)	4 (57%)
BSA (m^2)	1.81±0.03	1.85±0.03	1.83±0.08
systolic BP (mm Hg)	119±2	119±2	119±3
diastolic BP (mm Hg)	71±1	70±1	66±4
Septum (mm)	19±1	18±1	23±3
Posterior wall (mm)	10.0±0.2	10.0±0.2	9.9±0.1
LVIDd (mm)	43±1	45±1	45±2
LVIDs (mm)	25±1	26±1	26±2
LA (mm)	39±1	40±1	40±2
OT gradient (mm Hg)	14±2	9±2	19±13
$LVWT_{max}$ (mm)	22±1	21±1	24±3
LV mass (g)	188±9	186±10	198±41
EDV (ml)	104±3	110±3	98±7
ESV (ml)	27±1	28±1	25±3
EF (%)	71±1	70±1	67±3
SV (ml)	77±2	82±3	73±6
$LVWT_{max}$-I (mm/m^2)	12.0±0.3	11.7±0.4	13.1±1.6
LV mass-I (g/m^2)	100±4	99±5	105±19
EDV-I (ml/m^2)	57±1	59±1	54±4
ESV-I (ml/m^2)	15±1	15±1	14±2
SV-I (ml/m^2)	43±1	44±1	40±3

Table 6-5: Clinical characteristics of patients by ET-1 genotype

6.4. Discussion

The association of genetic polymorphisms with cardiovascular disease has been well established. The combination of these polymorphisms with genetically determined cardiovascular diseases such as HCM has also been explored and provides an attractive explanation for the wide phenotypic variability observed in HCM. Some investigators have reported that as much as 60% of the variation in LV mass in individuals is due to genetic factors. (Verhaaren *et al.* 1991) The association between particular genetic factors and HCM is further enhanced when there is a plausible theory as to the mechanism by which the polymorphism affects phenotypic expression.

The I/D polymorphism of the ACE gene is an excellent candidate modifier gene because of its known affects on the expression of ACE itself. Higher levels of a circulating ACE and hence AG II should have significant affects on the development of LV hypertrophy in HCM due to its direct trophic action on cardiomyocytes independent of its impact on vasomotor function. Our results confirm that the DD polymorphism is associated with a greater degree of LV hypertrophy in HCM. This has important ramifications. Assuming from this that development of LV hypertrophy in HCM is in part dependent on circulating ACE levels, then blockade of the conversion of AG I to AG II or the blockade of the action of AG II at its receptor should abrogate the development of hypertrophy. As there is continuous turnover of cardiac muscle there must be a continuous stimulus to maintain the cardiac hypertrophy and therefore blocking of ACE/AG II should also effect a reduction in LV mass over time.

However, contrary to previous reports we could find no correlation between the G8002A ET-1 genotype and degree of LV hypertrophy in our cohort of HCM patients.

6.4.1. Limitations

ACE and ET-1 levels were not available for correlation with indices of cardiac hypertrophy. However, the I/D polymorphism in the ACE gene has previously been associated with variation in ACE levels. Although there have been reports of an association between both of these polymorphisms and malignant arrhythmias in different clinical settings (Kozak *et al.* 2002; Marian *et al.* 1993), we did not have arrhythmia monitoring available for comparison in these patients.

6.5. Conclusion

These results suggest that the renin-angiotensin system is implicitly linked with the development of LV hypertrophy in HCM and thereby offers a target for therapeutic intervention. Although these results do not exclude the role of endothelin in the development of LV hypertrophy they do suggest that the G8002A ET-1 polymorphism is not an important factor in the phenotypic expression of HCM.

SECTION III: DISEASE MODIFICATION

7. Modification of the Renin-Angiotensin System in HCM

7.1. Introduction

The renin-angiotensin system (RAS) plays an important role in the development of LV hypertrophy in hypertension and may also contribute to the increased cardiac mass in HCM. (Lechin *et al.* 1995; Yoneya *et al.* 1995) In addition to the classical or endocrine RAS a local or paracrine/autocrine RAS may respond to localized stimuli and tissue requirements. (Bader *et al.* 2001; Varagic and Frohlich 2002) Mechanical stretch and autonomic activity activate cardiac RAS cascades, and many or all RAS components, including aldosterone, are produced *in situ.* (Bader *et al.* 2001; Balcells *et al.* 1997; Delcayre *et al.* 2000; Varagic and Frohlich 2002)

Several adverse cardiovascular effects of the RAS are attributed to angiotensin-II (AG-II) activity at the angiotensin receptor-1 (AT_1). Activation of AT_1 receptors on fibroblasts, cardiomyocytes, smooth muscle cells and endothelial cells promotes mitogenesis, myocyte hypertrophy, myocardial fibrosis, vasoconstriction and arrhythmogenesis. (Berry *et al.* 2001; Paradis *et al.* 2000) In contrast, AG-II) activity at angiotensin receptor-2 (AT_2) receptors is reported to oppose some or all the effects of AT_1 activity. (Brede and Hein 2001; Kurisu *et al.* 2003) AT_1 receptor antagonists do not inhibit AT_2 receptors, and may actually augment their activity by increasing AG-II availability. (Berry *et al.* 2001) This, along with findings suggesting that local RAS production of AG-II may occur independently of renin and ACE activity, suggests that the optimal inhibition of local cardiac RAS requires combination therapy. (Balcells *et al.* 1997)

Animal models and clinical studies of hypertensive cardiomyopathy have demonstrated that ACE, AT_1 and aldosterone inhibition ameliorate myocardial fibrosis myocyte hypertrophy, perfusion abnormalities, and electrocardiographic LV hypertrophy. (Brilla *et al.* 2000; Frohlich 2001; Linz *et al.* 1995; Mathew *et al.* 2001; Pitt 1998; Sato *et al.* 2002; Varagic *et al.* 2001) Observations that these effects did not correlate with the activity of circulating RAS components or with the magnitude of blood pressure reduction suggested a role for local RAS or aldosterone in the pathogenesis of the cardiomyopathy. (Pitt *et al.* 2003)

LV hypertrophy in HCM develops primarily as a maladaptive consequence of mutant gene expression of sarcomeric proteins rather than to pressure or volume overload. (Thierfelder *et al.* 1994) HCM is similarly characterized by myocyte hypertrophy and disarray, and hyperplasia of several other cell types including fibroblasts, endothelial cells and smooth muscle cells. (Ferrans 1998) Myocardial fibrosis is common, and is often more pronounced than in hypertensive cardiac hypertrophy. (Tanaka *et al.* 1987)

Evidence that renin-angiotensin-system activity contributes to the severity of HCM has been suggested by observations that in comparison to the ACE gene intronic polymorphism, the intronic deletion polymorphism is associated with increased plasma ACE concentrations and occurs at an increased frequency in HCM patients than in unaffected family members or in unrelated controls, and may also influence the severity of LV hypertrophy. (Lechin *et al.* 1995; Marian *et al.* 1993; Pfeufer *et al.* 1996; Tesson *et al.* 1997) Common polymorphisms in both AT_1 and AT_2 receptors may also influence the magnitude of hypertrophy. (Deinum *et al.*

2001; Osterop *et al.* 1998) Finally, in a mouse model of human sarcomeric gene HCM, AGII blockade reduces the degree of myocardial fibrosis. (Lim *et al.* 2001)

The clinical utility of RAS system manipulation in HCM remains clinically untested despite its gradual introduction as an empiric therapeutic modality. The primary hypothesis of this study is that the RAS is involved in the genesis and maintenance of LV hypertrophy in HCM, and ACE inhibitors and/or AT1 receptor antagonism will cause regression of the increased LV mass, leading to improved symptoms and functional indices.

7.2. Methods

This double-blind, placebo-controlled study was performed after informed consent according to a protocol approved by the Intramural Research Board of National Heart, Lung, and Blood Institute, National Institutes of Health. The *primary goal* of this study was to determine whether six months of ACE inhibition and/or AT_1 receptor blockade therapy reduces cardiac hypertrophy compared to placebo in patients with HCM.

One hundred and thirty-one consecutive unrelated patients were evaluated. Forty-two subjects did not meet entry criteria. **Table 7-1 and 7-2.**

Inclusion Criteria:
age 20-55 years
LV wall thickness ≥16 mm by MRI
LV outflow tract gradient of ≤30 mm Hg at rest, and ≤55 mm Hg following isoprenaline infusion to a heart rate of 120 bpm at cardiac catheterization
NYHA functional class I-II.

Table 7-1: Inclusion criteria in enalapril/losartan study

Exclusion Criteria:
significant other cardiovascular disease
chronic atrial fibrillation
bleeding disorder or anaemia
renal impairment (urea > 22 mg/dl and creatinine >1.4 mg/dl); K^+ <3.3 mmol/l or >5.1 mmol/l
hypertension: >160 mm Hg systolic or >95 mm Hg diastolic
hypotension: systolic arterial pressure <100 mm Hg confirmed 30 minutes later
pregnancy and lactation
LV ejection fraction <50% (radionuclide angiography)
dependence on diuretics, verapamil, β-blockers, or anti-arrhythmic drugs
sensitivity to ACE inhibitor e.g. angio-oedema
ACE inhibitor or losartan treatment within 6 months of the study

Table 7-2: Exclusion criteria in enalapril/losartan study

The remaining 89 patients were randomized to receive placebo, 10 mg enalapril, 50 mg losartan, or 10 mg enalapril plus 50 mg losartan. Block randomization was performed separately for men and women. Investigators were blinded to treatment assignment.

Patients underwent echocardiography, cardiac MRI, treadmill exercise test, ergometric upright bicycle exercise testing with breath-by-breath gas analysis, ambulatory ECG Holter monitoring, radionuclide angiography, and cardiac catheterisation. All studies were performed at baseline and at 6 month follow-up.

Patients in the 4 arms of the study were well matched with regards to clinical findings and severity of HCM. In addition components of the RAS were also assessed including ACE genotype. **Table 7-3.**

	Placebo n=22	Losartan n=21	Enalapril n=23	Combination n=23
Age (years)	34±9	34±9	38±8	37±11
Gender (males)	14 (64%)	14 (67%)	16 (70%)	14 (61%)
NYHA	1.7±0.1	1.6±0.5	1.7±0.1	1.8±0.4
Symptoms				
nil	2 (9%)	2 (10%)	5 (22%)	4 (17%)
angina	9 (41%)	5 (24%)	8 (35%)	9 (39%)
dyspnoea	14 (63%)	14 (67%)	12 (52%)	15 (65%)
pre-syncope	7 (32%)	8 (38%)	7 (30%)	11 (48%)
syncope	1 (5%)	3(14%)	5 (22%)	4 (17%)
palpitations	14 (64%)	8 (38%)	12 (52%)	12 (52%)
fatigue	3 (14%)	3 (14%)	2 (9%)	7 (30%)
Echocardiogram				
septum (mm)	17±1	17±1	17±1	17±1
posterior wall (mm)	10±1	9±0.2	9±0.4	9±0.2
LVIDd (mm)	44±1	45±1	48±1	44±1
LVIDs (mm)	25±1	26±1	27±1	25±1
LA (mm)	37±1	44±1	44±1	41±2

Table 7-3: Baseline characteristics of patients in enalapril/losartan study

7.2.1. Radionuclide Ventriculography

LV systolic function was determined by estimating rest and exercise LV ejection fractions. Diastolic function was determined by measurements of peak filling rate (PFR), time to peak filling rate (TPFR), and atrial contribution to LV stroke volume. (Bonow 1990)

7.2.2. Cardiac Catheterisation

Cardiac catheterisation was performed via the right femoral artery and vein. Selective coronary angiography was performed at baseline to exclude existing significant coronary artery disease. This was not repeated at follow-up. The presence of LV outflow tract obstruction was excluded by recording simultaneous LV and femoral artery pressures. To ensure there was no provocable LV outflow tract obstruction, patients received an isoprenaline infusion to a heart rate of approximately 120 bpm while recording from LV and femoral artery.

7.2.3. Renin-Angiotensin System

ACE genotype was determined by previously described methods (section 6.2.1.). Blood for renin, ACE, and AG-II levels was collected after >15 minutes lying supine and after an overnight fast. Blood was spun down immediately, and serum was taken on wet ice directly to the Clinical Pathology Lab for analysis.

7.2.4. Safety Considerations

As enalapril and losartan are vasodilators and may therefore increase LV systolic load by aggravating LV outflow obstruction, only patients with non-obstructive HCM were included in the study. Following randomization, heart rate

and blood pressure were monitored for 3 days on drug therapy. In the absence of an adverse event, the patient received the drug for six months. Haemoglobin, platelets, coagulation, electrolytes, renal function and liver function were checked at baseline and at 1, 3, and 6-month follow-up. Urinary pregnancy test was performed monthly in women of child-bearing age.

7.2.5. Statistics

Data are expressed as mean ± 1 SEM. Data were compared using Student t test. Pearson's test was used to examine the correlation between variables. A two-sided $p<0.05$ was considered significant. Statistical significance was tested following Bonferroni correction for multiple comparisons.

7.3. Results

7.3.1. Regression of LV Mass and Symptoms

LV mass decreased significantly in the enalapril and enalapril plus losartan arms of the study compared to the placebo arm. There was a smaller insignificant decrease in LV mass compared to placebo in the losartan arm of the study. LV diastolic, systolic and stroke volumes were unaffected in any of the treatment groups.

Table 7-4.

	Placebo	Losartan	Enalapril	Combination
Baseline LV mass (g)	235±19	237±21	241±14	214±21
Follow-up LV mass (g)	234±16	230±22	225±14	196±19
Δ LV mass (g)	-1.3±6	-6.7±4	-16.5±4*	-18.2±5*
Δ EDV (ml)	-5.3±3	2.5±5	6.4±4	-2.0±3
Δ ESV (ml)	-0.6±2	0.5±4	-0.8±2	-0.7±2
Δ SV (ml)	-4.7±3	2.3±3	-5.6±3	-1.3±3
*p<0.05 compared to controls				

Table 7-4: Change in MRI indices

Proximal septal thickness by echocardiography was reduced significantly in the enalapril arm of the study. Left atrial dimension was also significantly diminished in patients on enalapril plus losartan. Other LV indices were unaffected by ACE inhibition or AT_1 antagonism. **Table 7-5.**

	Placebo	Losartan	Enalapril	Combination
Δ septum (mm)	0.2±0.2	-0.1±0.3	-0.5±0.2*	-0.3±0.2
Δ posterior wall (mm)	-0.1±0.1	-0.2±0.2	0.1±0.2	0±0.1
Δ LVIDd (mm)	-0.4±0.3	1.0±0.8	-0.6±0.5	-0.7±0.3
Δ LVIDs (mm)	0.4±0.3	0.7±0.5	0.7±0.3	-0.3±0.4
Δ left atrium	-0.4±0.5	0.3±0.5	-0.7±0.4	-1.3±0.4**
*p<0.01, **p<0.001 compared to placebo				

Table 7-5: Change in echocardiographic indices

The New York Heart Association functional class improved significantly in the enalapril plus losartan arm of the study (from 1.8±0.4 to 1.5±0.5, p<0.02) but not in the other arms of the study. There was no significant change in individual cardiac symptoms.

7.3.2. Clinical Parameters

Exercise Testing: Treadmill exercise testing was not associated with any complication. No significant changes in heart rate and systolic and diastolic blood pressures were noted at rest or with exercise in any of the study-arms. Similarly, exercise durations were not changed significantly. **Table 7-6.**

	Placebo	Losartan	Enalapril	Combination
Maximum HR (bpm)	0±2	12±8	7±9	7±9
Peak sBP (mm Hg)	-2±6	10±11	13±9	7±9
Peak dBP (mm Hg)	5±5	10±6	4±4	6±6
Duration (sec)	-21±35	40±39	15±47	-9±30

Table 7-6: Changes in exercise parameters

Cardiac Catheterization: Most of the haemodynamic changes occurred in the enalapril plus losartan arm of the study and were limited to modest changes in systolic and mean arterial pressures and LV end-diastolic pressure. Notably, the absence of significant LV mass reduction in patients receiving losartan occurred despite significant reduction in systolic aortic pressures (15 ± 12 mm Hg, $p<0.01$), a change that was greater than that observed in patients receiving enalapril (7 ± 14 mm Hg). LV outflow tract pressure gradient did not increase significantly in any therapeutic arm of the study. Cardiac output and pulmonary arterial capillary wedge pressure were unchanged in the placebo group over the 6-months period, and did not change significantly in the three treatment groups compared to placebo. **Table 7-7.**

There was no significant correlation between changes in arterial pressures and LV mass regression.

	Placebo	Enalapril	Losartan	Combination
Heart rate (bpm)	-1±3	1±3	1±3	-2±2
RA mean (mm Hg)	0±1	0±1	0±1	1±1
RV EDP (mm Hg)	0±1	0±1	0±1	0±1
PA mean (mm Hg)	0±1	0±1	-1±1	2±1
PACW mean (mm Hg)	0±1	0±1	-1±1	1±1
Cardiac Index (L/min/m^2)	-0.1±0.1	0.1±0.1	-0.3±0.1	0±0.1
Ao systolic (mm Hg)	2±3	7±3	15±3**	17±4**
Ao diastolic (mm Hg)	-1±2	5±2	4±2	8±2***
Ao mean (mm Hg)	-1±2	4±3	4±2*	14±3***
LV systolic (mm Hg)	1±3	10±2*	9±3	19±3***
LV EDP (mm Hg)	0±2	1±2	0±2	5±1
LV OT gradient (mm Hg)	-2±2	1±2	-3±2	3±4

Data represent mean ± SEM change from baseline to follow-up; EDP, end-diastolic pressure; PACW, pulmonary artery capillary wedge, Ao, aorta; *p<0.05, **p<0.01, ***p<0.001 compared to placebo

Table 7-7: Changes in haemodynamic parameter

Radionuclide Ventriculography: There was no significant change in the indices of LV systolic and diastolic function at rest or with exercise during the 6-months of follow-up in any of the therapeutic arms of the study. **Table 7-8.**

	Placebo	Enalapril	Losartan	Combination
Resting EF (%)	-1±2	2±2	-1±2	-1±2
Exercise EF (%)	-6±1	4±2	1±2	2±2
TPFR (ms)	3±9	4±10	15±8	3±13
Atrial contribution to SV (%)	8±1	-1±2	0±2	-1±2

Table 7-8: Changes in radionuclide angiography parameters

7.3.3. ACE Genotype and Circulating RAS Hormones

The frequencies of the ACE D and I alleles were 0.64 and 0.36 respectively. These frequencies were not significantly different than those reported in large non-HCM populations. (Lindpaintner *et al.* 1996)

Plasma ACE levels varied markedly in the HCM population (2-85 U/L). DD genotype was associated with significantly higher serum ACE levels compared to both ID and II genotypes (plasma renin, AG-I, and AG-II levels were unaffected). **Figure 7-1.**

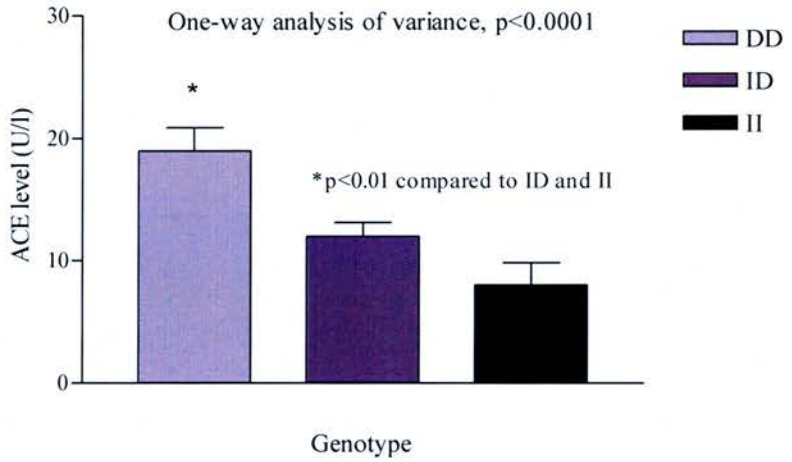

Figure 7-1: ACE levels by genotype

However, in this relatively small group of patients, neither LV mass nor $LVWT_{max}$ was affected by ACE genotype. In addition, there was no correlation between circulating RAS hormone concentrations (ACE, plasma renin, and AG-II) and LV mass or $LVWT_{max}$. **Figure 7-2 and 7-3.**

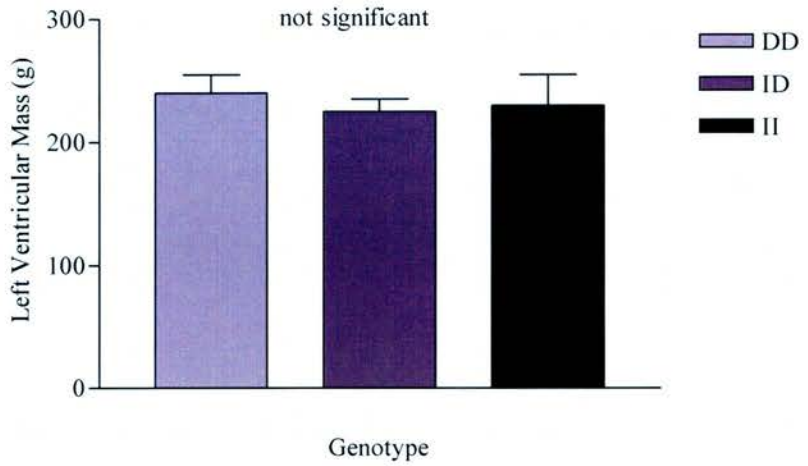

Figure 7-2: Affect of ACE genotype on LV mass

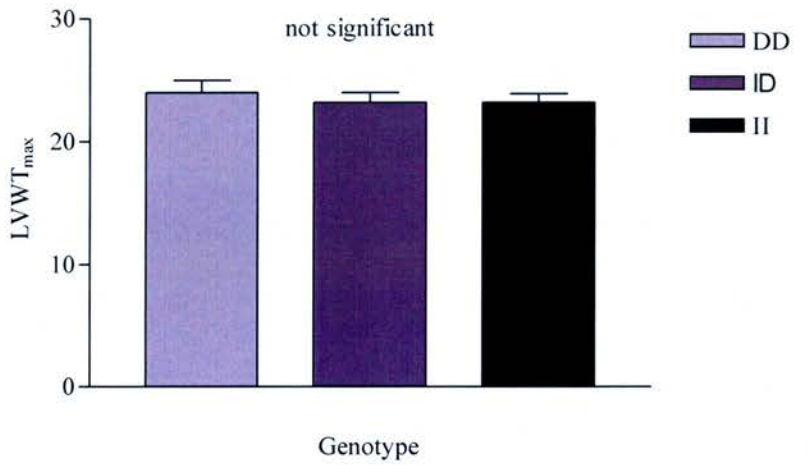

Figure 7-3: Affect of ACE genotype on $LVWT_{max}$

Changes in LV mass were unaffected by ACE genotype, as demonstrated by similar LV mass reduction in the enalapril plus losartan arm of the study in patients with the DD genotype and patients with ID or II genotype (19±8 gram and 18±8 gram respectively, p=0.89)

Changes following inhibition of the renin-angiotensin system: Enalapril, alone or in combination with losartan, significantly reduced plasma ACE and increased plasma renin concentrations. No significant changes in ACE levels were observed with losartan or placebo. **Figure 7-4.**

The changes in the levels of circulating ACE and other hormones of the RAS did not correlate with changes in LV mass and other cardiac indices.

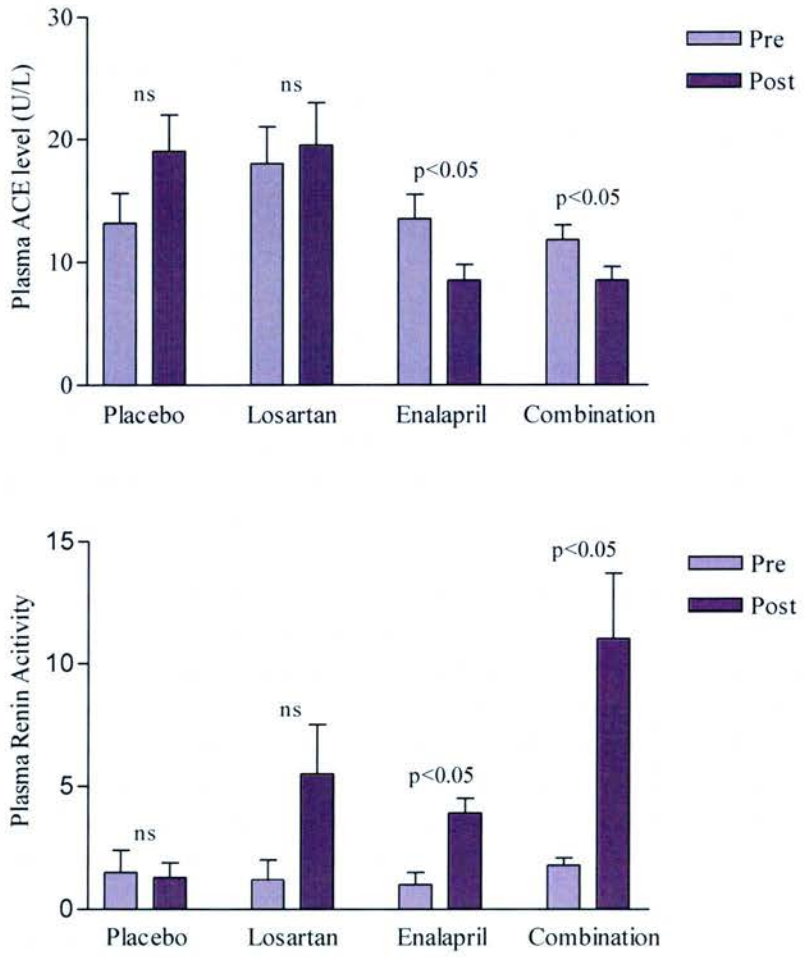

Figure 7-4: Change in plasma ACE concentration and plasma renin activity following RAS inhibition

7.3.4. Influence of Gender and RAS on Cardiac Indices

The frequencies of the ACE D and I alleles in men (0.62 and 0.38) and women (0.68 and 0.32) were similar. Women had lower LV mass and volumes before and after correcting for BSA.

In patients receiving enalapril or enalapril plus losartan, LV mass reduction was less in women compared to men but the difference was not significant (13±4 gram versus 21±5 gram, p=0.2).

7.3.5. Adverse Events and Long Term Outcome

Therapy was terminated after 1.3-4.2 months in 7 patients due to adverse events detailed below. (More than one adverse event per patient) **Table 7-9.**

	All patients	Placebo	Enalapril	Losartan	Combination
Hypotension	5	0	1	1	3
Light-headedness	5	0	1	1	3
Fatigue	3	0	1	0	2
Cough	1	0	1	0	0
Tachycardia	1	0	0	0	1
Hearing Loss	1	1	0	0	0
Total	16	1	4	2	9

Table 7-9: Adverse events during enalapril/losartan study period

Patients have been followed-up for a mean period of 4.1±1.5 years, maximum 8 years. Following participation in the study 34 patients did not receive long term medical therapy. The remaining patients were treated with β-blockers (12), verapamil (5), disopyramide (1), and/or enalapril or losartan (42). One patient in the placebo arm of the study died suddenly during strenuous exercise 7 years after completing the study.

7.4. Discussion

This is the first randomized placebo-controlled study of the effects of inhibition of the RAS in human HCM. Magnetic resonance imaging was used to determine LV mass to overcome a common problem associated with assessment of LV hypertrophy in HCM. Enalapril was tolerated well in most patients with non-obstructive HCM, and therapy for 6 months resulted in 16.5 grams of LV mass regression. Combination therapy with enalapril and losartan resulted in a similar reduction (18.2 grams) of LV mass, but was associated with a higher incidence of adverse events. Losartan reduced LV mass insignificantly (6.7 gm, $p>0.05$) despite larger mean changes in blood pressure than seen with enalapril. These effects of RAS inhibition in HCM are supported by similar findings in animal models of HCM. (Lim *et al.* 2001)

Our findings support the hypothesis that RAS activity contributes significantly to expression of phenotype in HCM. Many studies examining the role of RAS inhibition in patients with LV hypertrophy resulting from essential hypertension have demonstrated the effectiveness of ACE-inhibitors, AT_1 receptor

blockers and aldosterone inhibitors at reducing LV mass independently of blood pressure reduction. (Mathew *et al.* 2001)

As reported by others, we observed an increase in plasma ACE hormone levels associated with the ACE D allele. However, the changes in LV mass were not associated with circulating renin-angiotensin hormones, blood pressure response, or genotype. This unexpected finding may be attributed to the varied genetic causes of HCM in subjects in our study compared to studies examining for ACE polymorphism effects in single large pedigrees (Mathew *et al.* 2001; Tesson *et al.* 1997) and the relatively small number of patients included in the study.

As AT_1 receptors are believed to mediate many of the adverse effects of RAS and receptor blockade reduces cardiac mass in hypertensive LV hypertrophy and in various animal models, the absence of a significant effect of losartan on LV mass, was an unexpected finding. Several explanations could account for this. An effect on LV hypertrophy smaller than that of enalapril may be detectable by a larger study. Similarly, the use of larger doses of losartan may have improved efficacy. However, the losartan dose used here is comparable to those used in similar studies of hypertensive LV hypertrophy, and the hypotensive effects of losartan were similar to those of enalapril in this study. The differences in LV mass regression may therefore represent a divergence of the anti-hypertensive and anti-hypertrophic effects of the two drugs. As ACE inhibition interrupts the RAS cascade more proximally, the effects of enalapril on downstream RAS targets, other that the AT_1 receptor, may have been responsible for these differences. ACE inhibition also inhibits the enzymatic breakdown of bradykinin, increasing the activity of this anti-hypertrophic species. (Pitt 1998) Also, AT_1 receptor blockade does not inhibit angiotensin-II

binding at the AT_2 receptor, which may even be potentiated by increased AG-II and the resultant increased AT_2 receptor activity may actually antagonize AT_1 receptor activity and promote the development of LV hypertrophy. (Inagami 1999; Pitt 1998; Senbonmatsu *et al.* 2003) The findings of our study are consistent with a report that losartan (25 mg/day) failed to influence exercise-induced LV hypertrophy that is considered to be dependent on the RAS. (Myerson *et al.* 2001)

The reductions in cardiac mass were modest and significant cardiac hypertrophy remained. Optimizing ACE inhibition with more prolonged therapy and/or combinations with aldosterone antagonists (Tsybouleva *et al.* 2004) may improve LV mass reduction. The doses used in this study were relatively small to minimise side effects. In addition tissue bound ACE inhibitors are now available which may have greater efficacy. However it is likely that trophic signals other than those generated through RAS activity are also important. Indeed, our study suggests that female gender, and hence oestrogen, may also play an important independent role in modifying the cardiac phenotype in HCM as indicated also by other investigators. (Deinum *et al.* 2001)

7.4.1. Clinical implications for the management of HCM

Therapy reducing LV mass is only of benefit if it is also associated with an improvement in symptoms and/or prognosis. In this respect, several clinically important issues are not resolved by our study. Considerations for patient safety included the selection of relatively asymptomatic study subjects in whom a treatment effect on functional status will be minimal. Similarly, the short duration of therapy and the low event rate do not allow assessment of prognostic impact.

Our results also cannot be extended to obstructive HCM as patients with resting or provocable LV outflow obstruction were excluded because the vasodilatory effects of enalapril and losartan may worsen obstruction and associated symptoms.

Patients with hypertensive LV hypertrophy have been shown to gain significantly clinically and prognostically from renin-angiotensin system antagonism, more so than with other anti-hypertensive agents despite the same degree of blood pressure reduction. In these patients the use of ACE inhibitors and/or losartan not only block the molecular messenger, in part responsible for the increased cardiac mass, but also treat the underlying cause (i.e. the hypertension). In HCM, however, although the molecular messenger is perturbed, the underlying cause (sarcomeric dysfunction) remains.

Future studies should focus on whether further regression of LV hypertrophy can be achieved with newer agents and/or more prolonged therapy, and to determine the long-term consequences of LV mass regression in patients with HCM.

SECTION IV: DISCUSSION AND REFERENCES

8. Discussion

HCM remains a complex clinical condition. Despite its relatively high prevalence, its genetic diversity and variability of clinical expression limit our ability to risk stratify patients satisfactorily and identify those at greatest risk of SCD and hence those that would benefit most from ICD therapy. Underlying this is our poor understanding of which factors are responsible for the observed phenotypic variability in the first place. An increased understanding of the mechanisms responsible for the LV hypertrophy and other clinical features would at least allow us to identify targets for disease modification.

Potential modifiers must be considered in relation to other factors that cannot be altered such as gender and age. Modifiers of phenotypic expression need not be pathophysiologic however. IGF-I at physiologic levels may modify the expression of HCM favourably and therefore caution should be applied when considering antagonising certain systems that logically should result in favourable ventricular remodelling. Although regression of LV hypertrophy has been demonstrated using the growth hormone release-inhibiting somatostatin analogue, octreotide, in a very small number of patients, it is unclear what long-term clinical implications the use of this treatment will have beyond regression of LV mass.

BNP, although not a modifier of LV hypertrophy, is a modifier of the physiologic response to the increased LV mass. Its level, however, correlates with the disease causing mutation rather than the degree of LV hypertrophy, with higher levels observed in patients with mutations associated with a high risk of ventricular

arrhythmias. Therefore BNP may be included in the armamentarium of risk factors for SCD in HCM.

Conversely other modifiers are clearly pathophysiologic, such as the ACE gene I/D polymorphism, which has been shown to have many adverse affects in a variety of clinical scenarios and exaggerates the maladaptive process in HCM. In this instance we have been able to demonstrate that modification of the RAS system can result in significant regression of LV hypertrophy. However, further work is needed to ensure that this favourably modifies the other pathophysiologic features of the disease, such as diastolic dysfunction, and in particular the propensity to arrhythmias and SCD. In addition, an alternative strategy would be to prevent progression of LV hypertrophy by introducing ACE inhibition or angiotensin blockade at a stage before development of the LV hypertrophy.

Fibrosis and fibre disarray also contribute significantly to pathophysiologic processes in HCM. Therefore LV regression alone may be insufficient to alter clinical outcomes in HCM without modification of these processes also. However it is possible that ACE inhibition and/or angiotensin receptor blockade does have an effect in modifying these processes as well. However these studies were unable to assess this.

8.1. Conclusions

Identification of modifying factors in the phenotypic expression of HCM helps in our understanding of the mechanisms which explain the marked variation observed. This may provide avenues for therapeutic intervention either in the regression or prevention of development of LV hypertrophy in HCM with potentially subsequent improvement in symptoms and prognosis.

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REVIEW

Efficacy of Implantable Cardioverter Defibrillator Therapy for Primary and Secondary Prevention of Sudden Cardiac Death in Hypertrophic Cardiomyopathy

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BEGLEY, D.A., ET AL.: Efficacy of Implantable Cardioverter Defibrillator Therapy for Primary and Secondary Prevention of Sudden Cardiac Death in Hypertrophic Cardiomyopathy. *Risk stratification and effectiveness of implantable cardioverter-defibrillator (ICD) therapy are unresolved issues in hypertrophic cardiomyopathy (HCM), a cardiac disease that is associated with arrhythmias and sudden death. We assessed ICD therapy in 132 patients with HCM: age at implantation was 34 ± 17 years, and 44 (33%) patients were aged ≤ 20 years. Indications were sustained ventricular tachycardia (VT) or cardiac arrest (secondary prevention) in 47 (36%) patients, and clinical features associated with increased risk for sudden death (primary prevention) in 85 (64%) patients. There were 6 deaths and 55 appropriate interventions in 27 (20%) patients during a mean follow-up period of 4.8 ± 4.2 years: 5-year survival and event-free rates were 96% ± 2% and 75% ± 5%, respectively. ICD intervention-free rates were significantly less for secondary than for primary prevention: 64% ± 7% versus 84% ± 6% at 5 years, P = 0.02. Notably, 59 of 67 events (cardiac arrest and therapeutic ICD interventions), or 88%, occurred during sedentary or noncompetitive activity. Incidence of therapeutic shocks was related to age but not to other reported risk factors, including severity of cardiac hypertrophy, nonsustained VT during Holter monitoring, and abnormal blood pressure response to exercise. ICD related complications occurred in 38 (29%) patients, including 60 inappropriate ICD interventions in 30 (23%) patients. However, 8 (27%) of the patients with inappropriate shocks also had therapeutic interventions. ICD is effective for secondary prevention of sudden death in HCM. However, selection of patients for primary prevention of sudden death, and prevention of device related complications require further refinement. (PACE 2003; 26:1887–1896)*

cardiomyopathy, genetics, sudden death, prognosis

Introduction

Hypertrophic Cardiomyopathy (HCM) is a genetic disease with a prevalence of 1 to 2 per 1000 in the general population. The predominant cardiac phenotype is left ventricular (LV) hypertrophy often with associated diastolic and systolic dysfunction.[1] Patients with HCM frequently have disabling symptoms and are prone to atrial and ventricular arrhythmias and sudden death.[1–2]

The cumulative annual incidence of sudden death in a general HCM population is 1%–2%. However, prognosis depends critically on the referral patient population. The calculated incidence of sudden death is also higher for most family-based studies in which the genetic status of the subjects has been determined.[3] Genetic, clinical, morphological, and hemodynamic heterogeneity add to the complexity of risk evaluation in an individual patient.[1–8]

Design advances have encouraged the use of implantable cardrovcrte defibralator (ICD) in recent years and several clinical trials have indicated that therapy prevents sudden death in patients with certain cardiac problems, such as ischemic heart disease. However, these devices have not been evaluated in HCM as part of prophylactic trials for several reasons, including relative rarity of the disease and difficulties in identifying patients who are at risk of sudden death. There are therefore few data about the efficacy of ICD therapy in HCM, and this aspect of management strategy for prevention of sudden death in HCM remains very much in research domain.[9–18]

Ventricular arrhythmia is probably the most common cause of sudden death in HCM.[19] ICD therapy therefore presents a reasonable prospect

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of improving prognosis in selected patients with HCM. The present study explores efficacy of this therapy in a relatively large number of patients with HCM who received ICD for primary and secondary prevention of sudden death.

Methods

The study population consisted of 132 consecutive patients with HCM who were followed at the National Institutes of Health (NIH), and who received ICD devices between May 1987 and March 2001, Table I. Indications for ICD therapy were secondary prevention of sudden death in 47 (36%) patients; cardiac arrest in 39 patients; and sustained VT in 11 patients (3 patients had both); clinical features associated with increased risk for sudden death (primary prevention: young age, nonsustained VT on Holter, atrial fibrillation, bradycardia, induced VT, severe LV hypertrophy, myocardial ischemia, cardiac arrest/syncope, abnormal blood pressure [BP] response to exercise, malignant family history of sudden death, mutation associated with poor prognosis, and/or LV ejection fraction < 45%.) in 85 (64%) patients. Risk factors included syncope or presyncope, young age, family history of sudden death, molecular defect associated with a poor prognosis, nonsustained VT, LV dysfunction, outflow obstruction, myocardial ischemia, marked left atrial enlargement, and atrial fibrillation. Thirteen patients received their initial device at the NIH, 74 patients were recommended to have an ICD by the NIH, and in 45 patients the decision was made by the referring center.

Table I.

Clinical Characteristics of the HCM Patients Treated with ICD Devices

Gender	
males	80 (61)
females	52 (39)
Age at diagnosis (years)	25 ± 17
range	1–68
Age at implantation (years)	34 ± 17
≤ 20	44 (33)
≤ 40	88 (67)
range	10–72
Clinical presentations	
cardiac arrest	39 (29)
sustained VT	11 (23)
syncope	52 (39)
presyncope	73 (55)
palpitations	74 (56)
chest pain	52 (39)
dyspnea	67 (51)
fatigue	34 (26)
Coronary artery disease	3 (2)
Obstructive HCM	50 (38)
Prior procedures	
LV myotomy and myomectomy	29 (22)
septal myectomy plus apical aneurysectomy	1 (0.8)
mitral valve replacement	3 (2)
dual chamber pacing	24 (18)
alcohol septal ablation	3 (2)
Echocardiographic findings	
interventricular septum (mm)	23 ± 8
posterior LV wall thickness (mm)	11 ± 3
diastolic LV internal dimension (mm)	44 ± 8
systolic LV internal dimension (mm)	26 ± 10
left atrial dimension (mm)	44 ± 9

() = per cent; LV = left ventricle.

Evaluations

Studies included echocardiography (n = 131), treadmill exercise tests (n = 112), ambulatory electrocardiographic (ECG) monitoring (n = 130), genetic screening (n = 83), exercise thallium scintigraphy (n = 115), cardiac catheterization (n = 124), and electrophysiological study (n = 97).

Definitions

HCM: HCM was defined as LV wall thickness > 13 mm in the absence of another cause for the increased cardiac mass, presence of LV outflow obstruction, and/or genetic information.

Obstructive HCM: LV outflow gradient ≥ 30 mmHg at rest or ≥ 50 mmHg following provocation maneuvers.

Severe LV hypertrophy: maximum LV wall thickness ≥ 30 mm.

Marked left atrial enlargement: diastolic left atrial dimension ≥ 50 mm.

VT: sustained VT was defined as VT of > 30 seconds duration or requiring rapid termination due to hemodynamic compromise.

Therapeutic ICD intervention: ICD discharge that was preceded by syncope, presyncope, and/or other cardiac symptoms, or sustained ventricular arrhythmia determined by interrogation of ICD. (ICD interventions were spurious if in the absence of stored electrograms, the event was not associated with cardiac symptoms, or if they were related to device/lead malfunction, sinus tachycardia or atrial arrhythmia.)

Abnormal blood pressure response to exercise: a decrease or less than 10 mmHg increase in systolic blood pressure at peak treadmill exercise.

ICD Devices

One-hundred-forty-eight devices were implanted in 132 subjects. Twenty-six devices were

epicardial systems. Approximately 40% had defibrillator capability only, and 60% were cardioverter defibrillator devices.

Statistics

Patient data are presented as mean ± 1 SD. Differences from mean values were compared by Student's *t*-test. Cumulative survival and therapeutic ICD intervention-free rates were determined by product-limit survival analysis using sudden death and appropriate ICD shocks, respectively, as time variables. Cumulative event-free rates were calculated using the Log rank test. A P value of < 0.05 was considered significant.

Results

Clinical Events

Survival

Six deaths occurred during a mean follow-up period of 4.8 ± 4.2 years (maximum, 0.1 to 16.3 years): 5-year survival rate for all patients was 96% ± 2% (Fig. 1A) Four deaths were cardiac and occurred in young patients. Two children, aged 10 (epicardial leads) and 14 years (endocardial leads) died in the immediate postoperative period, from low cardiac output and cardiac tamponade, respectively; a child aged 14 underwent cardiac transplantation for severe drug-refractory symptoms 15 months after receiving an ICD and died in the immediate postoperative period; and a 24-year-old patient died suddenly while riding a motor cycle 34 months following device implantation (cause not established). Two patients, aged 53 years and 74 years, died from noncardiac causes 8.3 years and 9.4 years following ICD therapy, respectively.

Therapeutic ICD Interventions

Fifty five appropriate ICD shocks occurred in 27 (20%) patients, 5-year intervention-free rate, 75 ± 5 per cent, Table II, Figure 1A.

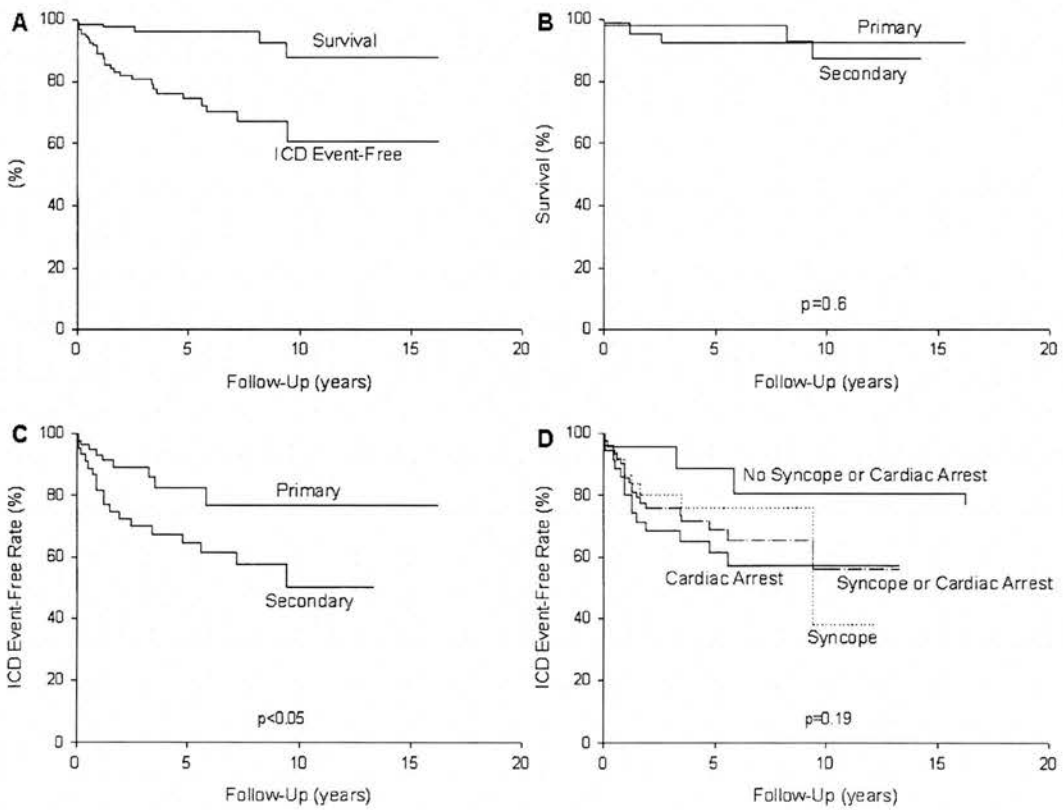

Figure 1. *Panel A. Survival rates and therapeutic ICD intervention-free rates for all of the patients; Panel B. Survival rates in patients in whom ICDs were implanted for primary and secondary prevention of sudden death; Panel C. Comparison of appropriate ICD intervention-free rates in patients in whom ICDs were implanted for primary and secondary prevention of sudden death; and Panel D. Comparison of ICD intervention-free rates in patients who presented with cardiac arrest, syncope, cardiac arrest and syncope, and patients who had neither presentations.*

Table II.

Results and Complications of ICD Therapy

Follow-up duration (years)	4.8 ± 4.2
range	0.1–16.3
Appropriate discharges	
patients	27 (20)
events	55
age at first event (years)	39 ± 21
range (years)	22–71
Patients with device related complications	37 (28)
Inappropriate ICD interventions	
patients	30 (23)
events	60
etiology-sinus tachycardia	15
- atrial tachycardia, atrial flutter, or atrial fibrillation	5
- lead malfunction/T wave oversensing	6
- nonsustained VT	2
- undetermined	2
Patients with inappropriate ICD shocks who also had therapeutic interventions	8/30 (27)
Miscellaneous associated problems	
high cardioversion defibrillation threshold	1
infection	4
anxiety/depression	5
direct ICD impact by baseball	1
cardiac transplantation	2
deaths	6
- operative	2
- noncardiac or following cardiac transplantation	3
- unknown	1

() = percent.

Table III.

Determined Circumstances Surrounding Cardiac Arrest Events* and Therapeutic ICD Interventions

Total Events	67
Sedentary	27 (40)
- sleeping/sitting/resting	23
- standing/talking	4
Noncompetitive activity	32 (48)
- running on the flat/jogging	8
- walking/running upstairs	7
- walking	4
- playing indoors	3
- school gymnastic activity	3
- fishing	2
- sexual intercourse	1
- surfing	1
- driving	1
- yard work	1
- postpartum	1
Competitive sport	8 (12)
- basketball	2
- soccer	2
- baseball	1
- frisbee	1
- pick up football	1
- tennis	1

Events prior, to, and events during follow-up; () = percent.

Circumstances Surrounding Cardiac Events

Table III details the circumstances surrounding cardiac arrest and appropriate ICD discharges. Notably, 59 of 67 (88%) of the events, occurred during sedentary or mild to moderate activity (undetermined in two cases). Only 8 (12%) events were related to strenuous competitive activity.

Primary Versus Secondary Prevention of Sudden Death

The duration of follow-up of patients who received ICD for primary prevention was significantly shorter than in patients who received devices for secondary prevention of sudden death (Table IV). The clinical characteristics of the two groups were similar—secondary prevention was associated with significantly higher number of risk factors per patient but the difference became insignificant when cardiac arrest was excluded as a risk factor.

Survival rate in the two groups were similar: 94% ± 3% for primary prevention versus 98% ± 2% for secondary prevention of sudden death, $P = 0.8$ (Fig. 1B). However, the cumulative therapeutic ICD intervention-free rate was significantly higher in patients in whom ICD therapy was for primary versus secondary prevention of sudden death: the 5-year event-free rates were 84% ± 6% and 64% ± 7% respectively, $P < 0.02$ (Fig. 1C).

Cardiac Arrest Survivors

The ICD intervention-free rate was also significantly higher in the 94 patients who did not present with aborted sudden death compared to that in the 38 cardiac arrest survivors: 87% ± 5% and 60% ± 9%, respectively at 5 years, $P = 0.026$.

Risk Factors for Therapeutic ICD Intervention

Young patients (<21 years) and older patients (>40 years) had higher rates of therapeutic ICD intervention compared to patients aged 21–40. (Fig. 2A). However, other risk factors such as severity of LV hypertrophy, LV outflow obstruction, nonsustained VT during ambulatory ECG

Table IV.

Comparison of Clinical Findings and Prevalence of Risk Factors in Patients who received ICD Therapy for Primary and Secondary Preventions of Sudden Death

	Prevention of Sudden Death		
	Primary	**Secondary**	**P value**
Number	85 (65)	47 (35)	
Follow-up duration (years)	3.4 ± 3.2	7.5 ± 4.2	< 0.0001
Gender (males)	52 (61)	28 (60)	NS
Symptoms of impaired consciousness			
syncope	38 (45)	14 (30)	NS
presyncope	48 (56)	25 (53)	NS
syncope and/or presyncope	66 (78)	29 (62)	NS
cardiac arrest, syncope, and/or presyncope	66 (78)	47 (100)	< 0.0001
Age (years)	34 ± 16	34 ± 20	NS
≤ 20	24 (28)	20 (43)	0.12
≤ 40	58 (68)	30 (64)	NS
Family history of sudden death	39 (46)	15 (32)	NS
>1 sudden death in first degree relatives	5 (6)	1 (2)	NS
Arrhythmia			
atrial tachycardia, flutter, or fibrillation	19 (22)	9 (19)	NS
spontaneous nonsustained VT	51 (60)	32/46 (70)	NS
exercise induced VT	2 (2)	3 (6)	NS
spontaneous sustained VT	0 (0)	11 (23)	< 0.0001
spontaneous nonsustained or sustained VT	51 (60)	36/46 (78)	NS
sustained VT induced at electrophysiological study	46/60 (77)	32/37 (86)	NS
Abnormal blood pressure response to exercise	21/83 (25)	13/37 (35)	NS
Severe LV wall hypertrophy	19 (22)	11/45 (24)	NS
Marked left atrial enlargement	20/84 (24)	10/43 (23)	NS
LV outflow obstruction	31/84 (37)	19/46 (41)	NS
Myocardial ischemia*	38/74 (51)	15/40 (38)	NS
LV dysfunction**	6 (7)	5 (11)	NS
Genetic defect associated with poor prognosis	8 (9)	1 (2)	NS
Number of risk factors per patient‡	3.9 ± 1.6	4.6 ± 1.7	0.025

() = percent; * = positive thallium scintigraphy or ischemic ECG changes during exercise or atrial pacing associated with chest pain/hypotension; ‡ = young age, nonsustained VT on Holter, atrial fibrillation, bradycardia, induced VT, severe LV hypertrophy, myocardial ischemia, cardiac arrest/syncope, abnormal BP response to exercise, malignant family history of sudden death, mutation associated with poor prognosis, and/or **ejection fraction < 45%.

monitoring, abnormal blood pressure response to exercise, and induced sustained ventricular arrhythmia were not associated with significantly higher rates of therapeutic ICD intervention (Fig. 2B–2F). ICD intervention rates were not higher in the patients who underwent LV myotomy and myectomy or alcohol septal ablation for obstructive HCM.

Genetic Tests

To date, molecular causes of HCM have been identified in 24 of the 85 patients who were tested: β-myosin heavy chain gene (*MYH7*) mutations in 19 patients; α-tropomyosin gene (*TPM1*) mutations in 1 patient; and cardiac actin gene (*ACTC*) mutations in 4 patients.

Complications

Serious complications were recorded in 38 (29%) patients, including 60 inappropriate shocks in 30 (23%) patients, (Table II). Notably, 8 (27%) of the 30 patients with inappropriate shocks also received therapeutic interventions. The complication rates were similar for primary and for secondary prevention of sudden death. Recurrences were prevented in most cases by reprogramming the devices.

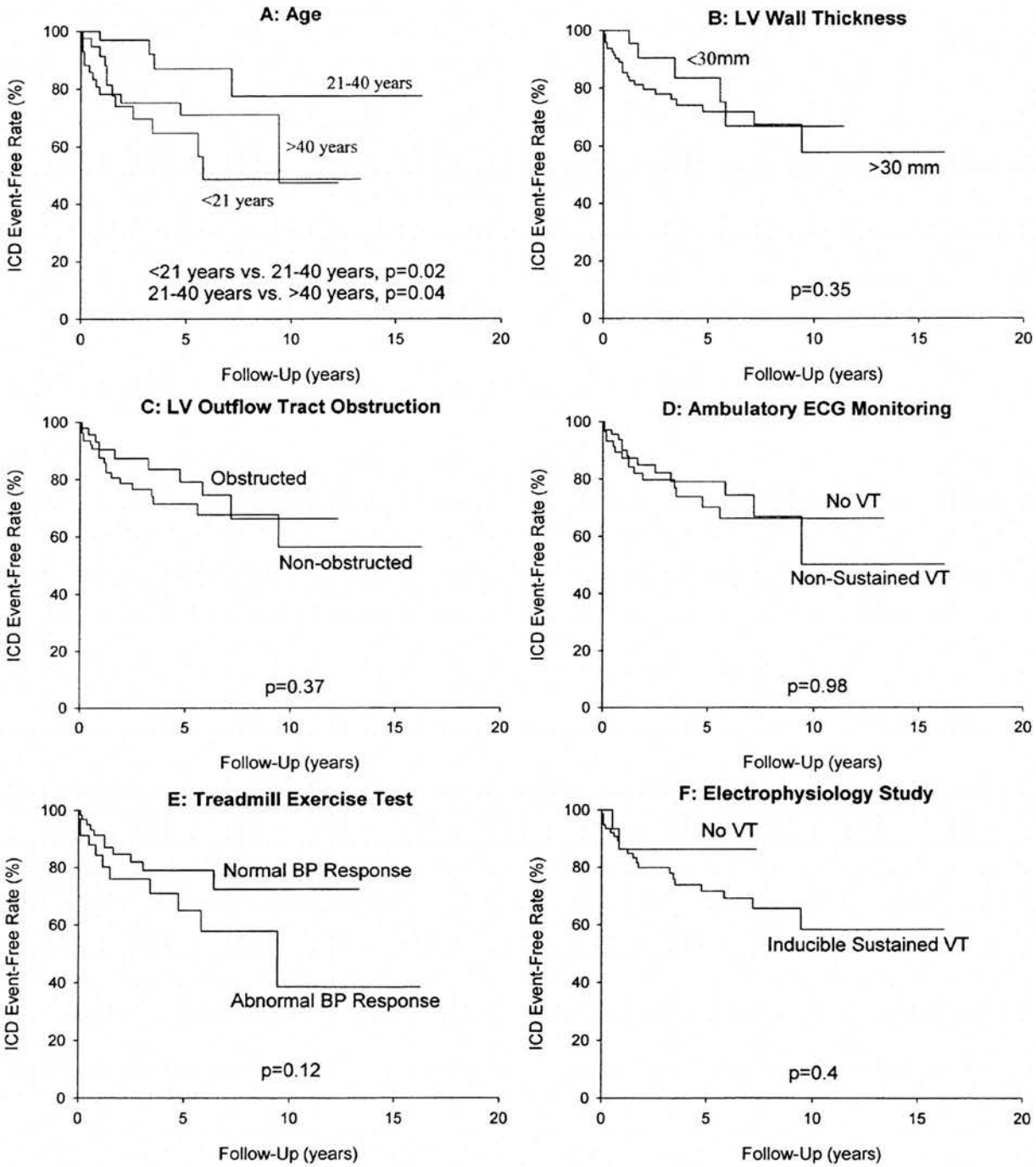

Figure 2. *The relation of therapeutic ICD intervention-free rates to 'risk factors' of sudden death: Panel A. Age; Panel B. Severity of LV wall thickness; Panel C. LV outflow obstruction; Panel D. Nonsustained VT during ambulatory electrocardiographic (ECG) monitoring; Panel E. Abnormal blood pressure (BP) response during treadmill exercise test (BP did not predict outcome also in the subset of subjects aged < 40 years); and Panel F. Induced sustained ventricular arrhythmia.*

Discussion

Significant advances in the design of ICD systems, with improved ease of insertion, longevity, programmability, and reduction in size, have lead to a more liberal use of the devices in HCM. However, there is still an important need to examine ICD therapy in HCM, as the decision represents a lifelong commitment to repeated procedures and significant exposure to potentially catastrophic complications.

There are scanty reports of ICD therapy in HCM involving mostly small numbers of highly selected patients.[9–18] Several of these describe its efficacy in aborted sudden death and in patients with sustained ventricular arrhythmia, indications that correspond to recommendations by NASPE and the ACC/AHA (Class 1 indication, general consensus that ICD indicated) guidelines.[20,21] Few reports describe ICD therapy for primary prevention of sudden death (AHA Class IIb indication, consensus that "usefulness/efficacy is less well established by evidence/opinion"). Some studies have noted a high incidence of therapeutic interventions in patients selected on the basis of sustained ventricular arrhythmia induced by programmed ventricular stimulation.[11–13] A recent study reported outcomes in 128 patients collected from 19 United States and European centers.[22] During a mean follow-up period of 3.1 years, 23% of patients received therapeutic interventions or antitachycardia pacing for ventricular arrhythmias. The event rate for 43 patients who were treated with ICD for cardiac arrest or sustained VT was 11% per year, and for the 85 patients who received devices for primary prevention the event rate was 4.5% per year.

Risk Stratification in HCM

In the past two decades several indicators of increased risk have also been identified. Unfortunately, most are controversial and have poor positive or negative predictive values.[23]

Young Age

The evolving cardiac disease state in the young is associated with an estimated 2% to 6% cumulative annual sudden death rate.[24–29] A third of the patients in the present study were <21 years of age. Young age was frequently associated with several additional risk factors. The present study suggests that the efficacy of ICD is most apparent in two age groups: patients aged <21 years and those >40 years.

Family History

Sudden death may occur even in family members in whom HCM is reportedly caused by "benign mutations." Further, a 'malignant' family history of sudden death originally referred to the occurrence of premature sudden death (aged <55 years) in two or more first-degree relatives.[30] Although a family history of sudden death was common in our patients, a 'malignant' family history was infrequent. Notably, it was also uncommon in the cardiac arrest survivors and in the patients who had therapeutic ICD interventions.

Genetic Causes of HCM

The severity of the clinical disease often varies significantly even in family members in who HCM is caused by the identical mutation.[3–8] For example, the mutation may be associated with no detectable disease in some family members but cause severe LV hypertrophy and disabling symptoms in other family members. The differences in phenotypic expression indicate the importance of modifying genes and genetic background. These findings further emphasize the need to individualize risk stratification even in patients in whom the genetic cause of the HCM has been determined. Additional problems relate to failure to identify the genetic cause in half of the cases of HCM, and availability of the prognostic significance of only a few of the described mutations.

Arrhythmias

Arrhythmias are common in HCM. Progressive left atrial enlargement associated with atrial fibrillation in about 10% of the patients, and may precipitate hemodynamic collapse. Nonsustained VT occurs in about 25% of patients, and has been considered an important marker of sudden death.[31,32] However, its prognostic significance, particularly in asymptomatic patients, has been questioned.[11,33]

Magnitude of LV Hypertrophy

A recent study reported that the magnitude of LV hypertrophy is directly related to the risk of sudden death.[34] It has also been noted that several malignant mutations are associated with severe LV hypertrophy and high disease penetrance, and conversely, HCM caused by some benign mutations is associated with mild cardiac phenotype and reduced disease penetrance.[7] However, other studies have demonstrated that LV thickness in patients who die suddenly is similar to survivors, with a great deal of overlap of LV wall thickness between the two groups.[35,36] Sinisterly, some genetic causes of HCM are associated with mild LV hypertrophy low disease penetrance but a high incidence of sudden death.[8] The present study did not find a higher prevalance of severe LV hypertrophy in cardiac arrest survivors. Severe cardiac hypertrophy was also not associated with increased rate of therapeutic ICD interventions.

Myocardial Ischemia

Myocardial ischemia may occasionally induce syncope or sudden death in the young.[37] In the present study, myocardial ischemia was not significantly associated with aborted sudden death, ventricular arrhythmias or sudden death.

Abnormal Blood Pressure Response to Exercise

We noted an abnormal blood pressure response to exercise in about one third of the patients.[38,39] This 'risk factor' was not associated with increased rate of therapeutic ICD intervention, and was not more prevalent in cardiac arrest survivors.

LV Dysfunction

Patients with impaired systolic function are prone to arrhythmias and sudden death. LV dysfunction was present in 11 (8%) patients, in the current series and 3 had appropriate ICD interventions.

Clinical Presentation

Aborted sudden death and syncope have been associated with 8% and 4% annual mortality, respectively.[1,40] Cardiac arrest and syncope were associated with increased frequency of ICD discharge but this finding did not reach statistical significance. A possible explanation may be that many factors that cause symptoms of impaired consciousness, notably outflow obstruction and arrhythmias, were addressed in these patiens.

Physical Activity

Competitive athletic activity has been associated with increased risk for sudden death.[41] Most of the cardiac arrests and appropriate ICD events in our study occurred during sedentary or mild to moderate activity. However, although only a minority of ICD interventions and sudden deaths occurred during strenuous activity, the percentage of time spent in strenuous activity is low, so the risk of an event during strenuous activity is certainly higher than during rest or moderate activity. Whether the advice traditionally given to patients with HCM not to take part in strenuous activity improves prognosis remains uncertain.

Primary Versus Secondary Prevention of Sudden Death

Patients who received ICD had a better prognosis and lower annual cumulative therapeutic interventions (primary prevention, 3% and secondary prevention, 7%) than reported previously.[22] The low rate of therapeutic ICD interventions occurred despite the fact that most patients who received the devices for primary prevention had a mean of about four risk factors for sudden death, and therefore, most likely represented a more severely affected patient population than would normally attend a primary center.

With the exclusion of cardiac arrest, the risk profile of patients who received an ICD for secondary prevention was similar to patients who received the devices for primary prevention of sudden death. The incidence of events in the latter group was, however, significantly less than that in patients who received the former group. This indicates that there are as yet other important risk factors that have not been identified.

Special Considerations Related to ICD Therapy in HCM

Current ICD devices have several capabilities that are potentially useful in HCM: (1) Treatment of sinus node disease and atrioventricular conduction abnormalities that are common and often aggravated by commonly prescribed drugs (β-blockers, verapamil, and antiarrhythmic drugs). Indeed, heart block is occasionally the cause of sudden death.[19] As patients with HCM often have diminished stroke volumes they can only augment their cardiac output in response to increased physiological demand by increasing their heart rate. Hence, provision of dual chamber pacemaker function allows management of bradyarrhythmias and chronotropic incompetence; (2) Short programmed atrioventricular intervals reduces LV outflow obstruction in some affected patients,[42] and (3) Certain ICD devices are capable of terminating atrial fibrillation.

Difficulties with ICD therapy in HCM include: (1) inability to precisely identify patients who are at high and low risk, hence, its redundancy—most patients who receive the devices never have a life-threatening arrhythmic event; and (2) high incidence of serious side effects, the most common being inappropriate ICD interventions. Some of the problems are in part related to the young age of the patients in HCM[43–48]—infections and lead related problems tend to be higher in the young compared to adults, possibly because children are more active and may be less vigilant with wound care. Young patients also tend to achieve high sinus rates with activity that may be misinterpreted by the ICD. Other contributing factors and events are: the frequency of atrial arrhythmias and nonsustained VT in HCM, high electrical voltages resulting in T wave sensing, and small cardiac chamber cavities predisposing to cross-talk.

Study Limitations

Use of symptoms preceding shock as surrogate for ventricular arrhythmia is unreliable, especially in this patient population. Stored electrograms

for verification rhythm prior to shock, available in newer devices, address this issue. Delivery of "appropriate" therapy does not equate with resuscitation from death.[49] The selection of patients for primary prevention as with previous studies was not controlled. The significance of the risk factors for sudden death in this highly selected patient group may be different from that in a general HCM-population. It is also likely that the risk of sudden death is less in the HCM populations that are seen by cardiac centers that do not specialize in this disease. Hence, the findings of studies that describe the effectiveness of ICD therapy in patients largely referred to tertiary centers should be interpreted with caution and may not apply to patients with HCM managed at primary centers. Further, the experience with ICD therapy in HCM remains limited, and until more data are available, indiscriminate use of these devices in HCM may result in a high incidence of complications in patient populations that may be at low risk for sudden death. The finding that ICD therapy may have a role in some patients is not a substitute for careful risk evaluation in an individual patient. Importantly, about one third of the subjects had serious complications and the therapy was not used in most of the patients who were considered to be at high risk for sudden death. The risk profile of patients who had cardiac events was also similar to that in patients who did not have appropriate device interventions. These findings suggest that risk stratification in HCM remains a major challenge and careful consideration should be given to selection of patients for ICD therapy.

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Dual Chamber Pacemaker Therapy for Mid-Cavity Obstructive Hypertrophic Cardiomyopathy

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BEGLEY, D., ET AL.: Dual Chamber Pacemaker Therapy for Mid-Cavity Obstructive Hypertrophic Cardiomyopathy. *Intracavitary LV obstruction is an important determinant of clinical outcome in hypertrophic cardiomyopathy (HCM). In a minority of patients the obstruction is at the level of the papillary muscles. Mid-cavity obstructive HCM may be associated with a distal LV aneurysm and a worse prognosis. It is often not amenable to standard cardiac surgery for LV outflow obstruction. The long-term effects (mean follow-up 4.8 ± 2.9 years) of dual chamber (DDD) pacemaker therapy in 14 patients with mid-cavity obstructive HCM (mean age 34 ± 16 years, range 15–65 years) were studied. Patients were evaluated by cardiac catheterization at baseline and 6 months to 1 year after receiving DDD pacemakers off all drug therapy. Symptoms were improved in all patients and NYHA functional class reduced from 2.8 ± 0.1 to 1.9 ± 0.4 ($P < 0.0005$). Intracavitary LV pressure gradients was reduced significantly (43 ± 36 vs 84 ± 31 mmHg at baseline, $P < 0.0005$). There was a significant associated reduction in apical LV systolic pressure (152 ± 37 vs 188 ± 34 mmHg, $P < 0.001$). In addition, there was a trend towards increased exercise tolerance (445 ± 123 vs 396 ± 165). Cardiac output and LV filling pressures were unchanged. In conclusion, chronic DDD pacing results in significant symptomatic and hemodynamic improvement in this uncommon but important subset of patients with obstructive HCM in whom the role of cardiac surgery is less well defined compared with the more typical outflow tract location of LV obstruction. (PACE 2001; 24:1639–1644)*

pacing, hypertrophic cardiomyopathy, mid-cavity obstruction

Introduction

Hypertrophic cardiomyopathy (HCM) is a genetic disease with an autosomal dominant pattern of inheritance characterized by left ventricular (LV) hypertrophy in the absence of another cause for the increased cardiac mass. The cardiac hypertrophy is associated with LV outflow obstruction in about 30% of the patients.[1] In the typical LV outflow obstruction the pressure gradients are generated by systolic anterior motion (SAM) of a mitral valve leaflet and its coaptation with the inward moving hypertrophied anterior interventricular septum. The LV outflow tract obstruction is an important determinant of clinical course and is often associated with disabling cardiac symptoms; chest pain, dyspnea, presyncope, syncope, palpitations, and excessive fatigue.[1,2] Cardiac surgery, including myotomy and myectomy (Morrow procedure), mitral valve plication, and mitral valve replacement of been performed in patients with obstructive HCM for many years.[3–11] More recently, dual chamber (DDD) pacemaker therapy and alcohol septal ablation have been investigated for relief of drug refractory symptoms in obstructive HCM.[12–20]

In some patients with obstructive HCM the maximum LV hypertrophy and pressure gradient are at the level of the papillary muscles. The mid-cavity obstruction may be associated with a distal LV aneurysm. This unusual variety of obstructive HCM is more commonly associated with certain molecular genetic defects.[21,22] In its pure form there is no SAM, but occasionally, there is obstruction at the LV outflow tract associated with SAM and at the mid-cavity level.[23,24] Patients with mid-cavity obstructive HCM are often symptomatic, are prone to ventricular arrhythmias arising from the distal LV aneurysm, and may have a worse clinical outcome.[24] Therapeutic options are unfortunately often limited.[25,26]

The present study investigates the ability of DDD pacemaker therapy to relieve symptoms and

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to reduce the intracavitary pressure gradients in patients with mid-cavity obstructive HCM.

Methods

Patients

The study population consisted of 14 patients diagnosed with mid-cavity obstructive HCM and severe symptoms refractory to standard medical therapy who were referred to the National Heart, Lung, and Blood Institute between June 1991 and Oct 1998. Their clinical characteristics are detailed in Table I. Informed consent was obtained in accordance with study protocols approved by the Institute Review Board. All patients had failed to respond to standard medical therapy with adequate doses of verapamil and a β-blocker.

Table I.

Clinical Characteristics of the 14 Patients with Mid-Cavity Obstructive HCM

Age (years)	34 ± 16
Gender (M:F)	7:7
Family history	
HCM	7
Sudden death	3
New York Heart Association Functional Class	
I*	1
II	1
III	12
Clinical presentation	
Dyspnea	13
Chest pain	10
Presyncope	11
Syncope	5
Palpitations	7
Ventricular tachycardia	4
Genetic defect	
Unknown	9
Essential light chain gene	
$Arg^{154}His$	1
β-myosin heavy chain gene	
$Arg^{249}Gln$	1
$Arg^{663}Gln$	1
$Arg^{719}Gln$	1
$Leu^{908}Val$	1
Echocardiogram	
Proximal septum (mm)	20 ± 5.0
Maximum LV wall thickness (mm)	24 ± 5.9
LV internal dimension in diastole (mm)	39 ± 5.0
LV internal dimension in systole (mm)	20 ± 5.0
Left atrium (mm)	47 ± 7.4
Estimated intra-cavitary gradient (mm Hg)	80 ± 34

*recurrent syncope. HCM = hypertrophic cardiomyopathy; LV = left ventricular.

At baseline, patients underwent the following investigations: 12-lead electrocardiograph (ECG), symptom-limited Bruce protocol exercise test, M-mode, two-dimensional and Doppler echocardiography, and right and left heart catheterization as described previously.[14] Patients underwent repeat investigations 6–12 months following pacemaker implantation. Baseline and follow-up investigations were performed 5 half-lives off any cardiac medications.

A significant gradient was defined as a gradient > 30 mmHg across the LV mid-cavity, measured under basal conditions at cardiac catheterization.

Implantation of Permanent DDD Pacing Systems

DDD permanent pacemakers were implanted in all patients using standard techniques with the ventricular lead positioned at the right ventricular apex. At the baseline study, optimal AV delay was determined by observing maximal ventricular preexcitation from the 12-lead ECG. After measurement of right and left heart pressures, the hemodynamic indices were remeasured during DDD pacing with short AV delays.[14]

Statistical Analysis

Data are expressed as mean ± 1 SD. Paired data were compared by Student's paired *t*-test. A value of $P < 0.05$ was considered significant.

Results

At baseline, eight patients had no evidence of associated LV outflow obstruction, but five patients had, in addition to mid-LV obstruction, significant LV outflow tract obstruction (SAM with brief to prolonged interventricular septal contact). Figure 1 illustrates echocardiographic findings in a patient with mid-cavity HCM without LV outflow obstruction. Figure 2 illustrates LV angiographic findings in patients with mid-cavity obstructive HCM.

Functional Improvement

All patients were alive during a mean follow-up period of 4.75 ± 2.9 years (range 7 months to 8 years). The New York Heart Association (NYHA) functional class was reduced from 2.8 ± 0.1 to 1.9 ± 0.4, $P < 0.0005$ (Fig. 3). No patient had syncope during follow-up. Exercise time increased from 396 ± 165 to 445 ± 123 seconds ($P = 0.14$).

Five patients currently require no medication. The remaining patients have been treated with atenolol, disopyramide, sotalol, diltiazem, verapamil, enalapril and/or diuretics for residual symptoms and paroxysmal arrhythmias.

Hemodynamic Changes

The hemodynamic changes following pacemaker therapy are summarized in Figures 4 and 5. At the baseline cardiac catheterization, the mean apical systolic pressure was 188 ± 34 mmHg which

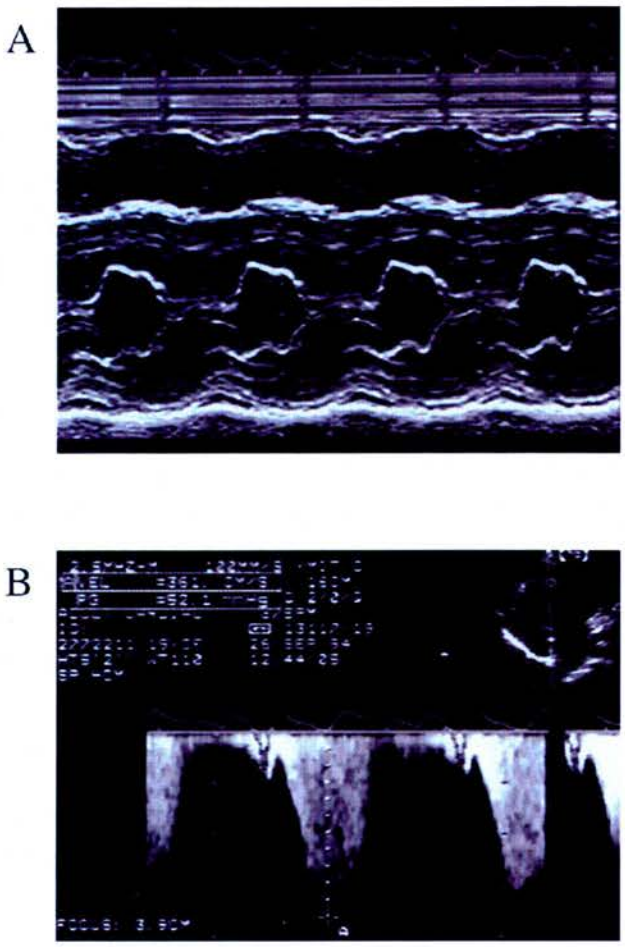

Figure 1. *(Panel A) Absence of left ventricular outflow tract obstruction (no systolic anterior motion [SAM]) in a patient with mid-cavity obstructive hypertrophic cardiomyopathy. (Panel B) Continuous wave Doppler interrogation of mid-cavity left ventricle demonstrating a velocity of 4.7 m/s indicating an intracavitary pressure gradient of about 87 mmHg.*

Figure 2. *Left ventricular (LV) angiographic appearance in a patient with mid-cavity obstructive hypertrophic cardiomyopathy (systolic frame). The end-hole pigtail catheter traversing the mid-cavity obstruction is seen. The marked hypertrophy divides the LV cavity into a proximal and a distal aneurysmal segment.*

decreased to 152 ± 37 mmHg at the follow-up evaluation, P < 0.001 ΣΦιγ> ÷Π. The mean intracavitary gradient decreased from 84 ± 31 to 43 ± 36 mmHg (P < 0.0001). The reductions in intracavitary pressure gradients in patients with pure mid-cavity obstructive HCM and in patients with obstruction at two LV sites are shown in Figure 5. Figure 6 illustrates the reduction in LV pressure gradients in a patient with mid-cavity obstruction only. Right heart pressures, LV filling pressures (pulmonary artery wedge pressure and LV end-diastolic pressure), and cardiac output did not change significantly.

Discussion

Mid-cavity obstruction is an uncommon phenotypic variety of HCM that may present at any

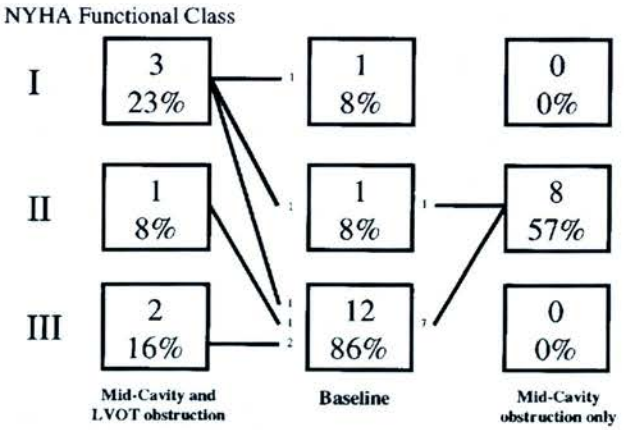

Figure 3. *Changes in New York Heart Association (NYHA) functional class status before pacemaker therapy (baseline) and at follow-up evaluation. Baseline characteristics are the central boxes. Follow-up functional class of patients with pure mid-cavity obstruction are given in the right-sided boxes and follow-up functional class of the remainder are given in the left-sided boxes.*

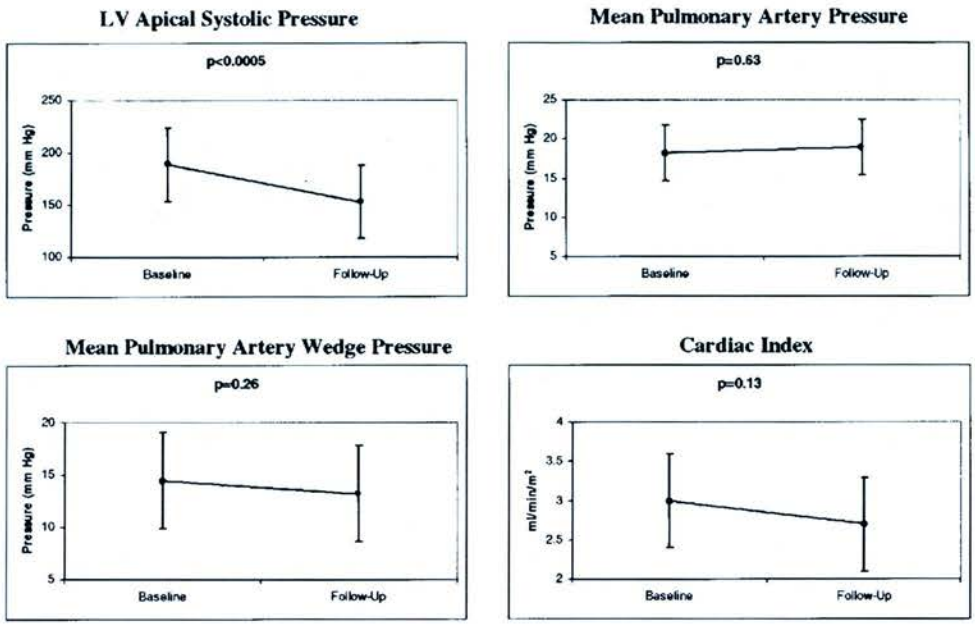

Figure 4. *Figure summarizing the hemodynamic findings before (baseline) and following (follow-up) DDD pacemaker therapy.*

age and is often associated with disabling symptoms, heart failure, and arrhythmias. Symptoms may respond to β-blockers and/or verapamil and judicious doses of diuretics.[27–31] Patients with drug-refractory symptoms present a difficult therapeutic challenge, particularly as the role of cardiac surgery for mid-cavity obstructive HCM is uncertain.

Numerous studies have demonstrated that DDD pacing relieves symptoms and reduces the more typical subaortic LV outflow gradients.[12–20] The present study suggests that DDD pacing may be also beneficial in mid-cavity obstructive HCM if symptoms continue to be disabling despite adequate pharmacotherapy. In this way, major surgery with uncertain outcomes or cardiac transplantation may be avoided.

In the present study, mid-cavity obstruction was complicated by LV outflow tract obstruction in 5 of 14 patients. However, relief of symptoms and reduction in LV intracavitary pressure gradients were similar in the patients with mid-cavity obstruction alone and in patients with two LV sites of obstruction.[32]

The mechanism by which DDD pacing reduces the intracavitary pressure gradient is uncertain. Due to markedly distorted LV morphology

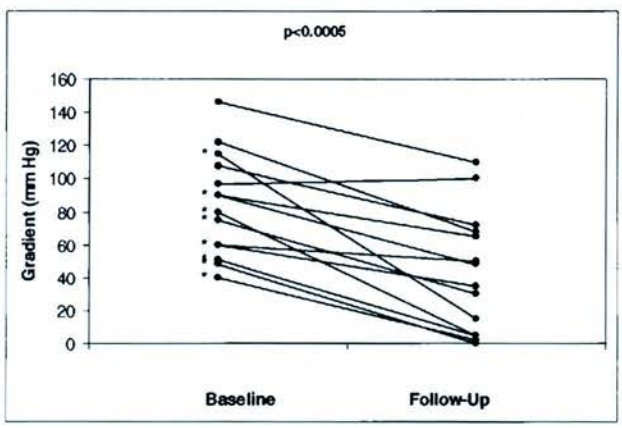

Figure 5. *Changes in intracavitary pressure gradients in individual patients. * = patients with mid-cavity obstruction but without significant left ventricular outflow obstruction.*

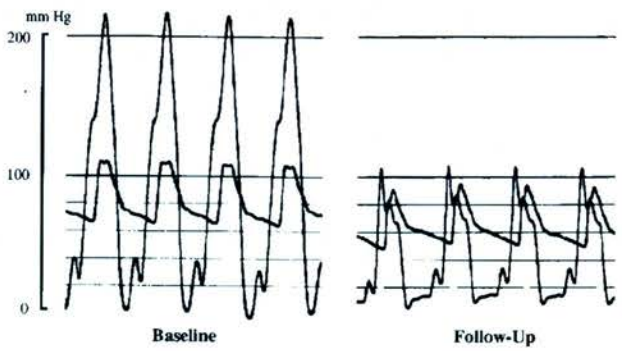

Figure 6. *Reduction in the intracavitary pressure gradient in a patient with mid-cavity hypertrophic cardiomyopathy.*

and limitations of echocardiography the authors could not ascertain that DDD pacing consistently widened the narrowed mid-cavity site. However, correct placement of the ventricular lead appears to be important for the success of the DDD pacemaker therapy. Initially, in two patients, screw-in leads were used to position the ventricular lead at the mid-septum (i.e., site of the obstruction). However, it became clear that maximum results are obtained when the ventricular lead is positioned at right ventricular apex as distal to the site of obstruction as possible.

A common presentation of mid-cavity HCM are symptoms of impaired consciousness caused by monomorphic ventricular tachycardia arising from the LV aneurysm (right bundle morphology with superior QRS axis).[33–36] The availability of defibrillators with DDD pacemaker capability has facilitated management of these patients, although the indication(s) for primary implantation of defibrillators in this patient population remains to be clarified.[37]

A second critical aspect of DDD pacemaker therapy is the programmed atrioventricular (AV) delay.[38,39] The AV delay should be short enough to allow for maximum ventricular preexcitation but sufficiently long not to interfere with left atrial emptying. Hence, the paced QRS duration should remain maximum during exercise tests, and Doppler interrogation of mitral inflow velocities at echocardiography should confirm adequate left atrial emptying during DDD pacing. Patients with delayed interatrial conduction delay (prolonged P wave) and rapid AV conduction present the greatest challenge. The addition of verapamil and/or a β-blocker drug in these patients may prolong AV node conduction and hence permit maximum preexcitation while maintaining left atrial contribution to cardiac output. Radiofrequency ablation or modification of the AV node will achieve the same result.

Study Limitations

This is a relatively small uncontrolled study of DDD pacemaker therapy. Indeed, two studies have suggested that some of the symptomatic benefits of DDD pacing may be due to a placebo effect.[40,41] One of the studies consisted of a small number of subjects,[41] and the other was associated with a high pacemaker complication rate.[42] The studies were not designed to rule out the possibility of a carryover effect and progressive changes that occur with prolonged pacing. A larger multicentric controlled study indicates that pacing has a profound and beneficial effect on hemodynamics as well as domains of quality-of-life.[42] An initial "placebo effect" was not observed with longer follow-up.[42] However, the present study does not support the use of DDD pacing for routine therapy of mid-cavity obstructive HCM. DDD pacing should be reserved for carefully selected patients with drug refractory symptoms, appropriate right ventricular anatomy, and suitable AV conduction during sinus rhythm.

Summary

In most patients with this rare morphological and hemodynamic variety of obstructive HCM that is often difficult to manage, DDD pacemaker therapy resulted in long-term improvement of symptoms and reduction of intracavitary pressure gradients without adversely affecting cardiac output or LV filling pressures.

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Utility of Genetic Screening in Hypertrophic Cardiomyopathy: Prevalence and Significance of Novel and Double (Homozygous and Heterozygous) β-Myosin Mutations

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ABSTRACT

Genetic screening of the β-myosin heavy chain gene (*MYH7*) was evaluated in 100 consecutive unrelated patients with hypertrophic cardiomyopathy (HCM) and 200 normal unrelated subjects. Seventeen β-myosin mutations were identified in 19 patients. Notably, 13, or 76%, were novel. Mutations were detected in both alleles in two patients: homozygous for $Lys^{207}Gln$ in one, and heterozygous for Pro^{211} Leu and $Arg^{663}His$ in another. No mutation was detected in the controls. *MYH7*-associated HCM was associated with more marked left atrial enlargement and syncope than non-*MYH7*-related HCM. Our findings indicate that: (1) screening methods should allow identification of novel mutations; and (2) more than one sarcomeric mutation may be present in a patient more commonly than is appreciated. Further studies are necessary to ascertain the clinical consequences of the novel and compound gene abnormalities, and to determine whether correlating functional domain to phenotype provides more useful information about the clinical significance of the molecular defects.

INTRODUCTION

HYPERTROPHIC CARDIOMYOPATHY (HCM) is a cardiac disease with an autosomal dominant pattern of inheritance, a variable phenotypic expression characterized by maladaptive left ventricular (LV) hypertrophy, and abnormalities of diastolic and systolic function (Wigle *et al.*, 1995). Affected subjects often have disabling symptoms and are prone to arrhythmias and sudden death (Chang *et al.*, 1995). HCM demonstrates nonallelic and allelic heterogeneity, and thus far, nine genes encoding sarcomeric components are known to cause HCM (Fananapazir, 1999; Towbin *et al.*, 2000; Mohiddin and Fananapazir, 2001). The most extensively studied gene is *MYH7*, coding for β-myosin heavy chain (Geisterfer-Lowrance *et al.*, 1990; Epstein *et al.*, 1992; Fananapazir and Epstein, 1994; Lankford *et al.*, 1995; Rayment *et al.*, 1995; Palmiter *et al.*, 2000). The familial HCM database lists 72 mutations in this gene that, by September 2001, were associated with HCM (FHC Mutation Database). Almost invariably, these are missense mutations affecting the motor or head-lever region of the molecule.

Determination of the molecular causes of HCM has enabled description of mutation-specific natural histories. For example, some β-myosin mutations are associated with high disease penetrance and a high incidence of sudden death; others with low penetrance and a relatively benign prognosis (Fananapazir and Epstein, 1994; Enjuto *et al.*, 2000; Richard *et al.*, 2000). Importantly, some sarcomeric mutations are associated with mild LV hypertrophy but a poor prognosis (Varnava *et al.*, 2001; Karibe *et al.*, 2001). It also allows for preclinical diagnosis in children and establishes the diagnosis in adults with mild disease. Hence, increasingly, a genetic diagnosis is considered an important component of the management of HCM. However, despite recent advances, the utility of genetic screening in individual patients is unclear.

It is also uncertain which screening method should be utilized. Mutations may be detected by: (1) specifically testing for each of more than 100 identified mutations; for example, by restriction fragment length polymorphism (RFLP); (2) sequencing the several hundred exons of these nine genes, with or without preliminary screening such as with single-strand con-

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formation polymorphism (SSCP) analysis; or (3) by studying suitably large kindreds using genetic linkage analysis. Following detection of novel mutations, it is then desirable to prove causality (even in genes known to cause HCM), and to determine the natural history of the associated HCM.

We determined the prevalence and diversity of *MYH7* mutations in a consecutive cohort of unrelated HCM patients and in normal controls to: (1) evaluate a sequencing-based strategy for routine mutation detection in unselected patients: (2) determine the degree of *MYH7* heterogeneity in normal individuals; and (3) evaluate the feasibility of describing the natural histories of the associated HCM.

METHODS

Patients

A total of 100 consecutive unrelated patients with HCM referred to the National Institutes of Health were studied. Informed consent was obtained under protocols approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute.

Clinical studies

Studies included 2-D echocardiography, 12-lead electrocardiography, and in selected cases, a treadmill exercise test. HCM was defined as LV wall thickness >13 mm in the absence of another cause for cardiac hypertrophy. A family history of HCM was defined as present if obtained at the initial clinic interview and does not include results of subsequent family screening. A family history of sudden death (SD) was defined as the sudden death of 1 or more first-degree family members under the age of 45 years.

Genetic analysis

Genetic analysis was performed on genomic DNA extracted from whole blood (Gentra). Using previously described primer pairs and methods (Fananapazir and Epstein, 1994), each of the first 23 exons of *MYH7* was amplified by PCR. SSCP was performed for each exon for every patient to identify anomalous conformers. Anomalous conformers were sequenced (BigDye Terminator, ABI Prism 310) to detect sequence abnormalities (SeqMan, DNAStar). RFLPs were predicted from mutant and wild-type sequences (MapDraw, DNAStar) and mutations confirmed by digestion when possible. Genomic DNA from 200 unrelated control individuals was also screened. For each novel mutation, 100 control DNAs were screened by RFLP. To determine the degree of *MYH7* heterogeneity in a normal population, a further 100 unrelated controls were screened by SSCP for each exon in which a mutation had been detected in HCM patients.

Statistics

Data are expressed as mean ± SD. Continuous variables were analyzed using Student's *t*-test for unpaired data. Noncontinuous variables were analyzed using Fisher's exact test. A p value of <0.05 was considered significant.

RESULTS

Prevalence of known and novel β-myosin mutations in the HCM population, and genotype–phenotype correlations

Results of the genetic analysis in the 100 patients with HCM and their clinical findings are presented in Tables 1 and 2, re-

TABLE 1. β-MYOSIN (*MYH7*) MUTATIONS IDENTIFIED IN 100 UNRELATED PATIENTS WITH HCM

Patient	*Mutation*	*Exon*	*Charge change*	*Morphology*	*Clinical findings*
1	$Arg^{143}Gly$	5	Positive-neutral	ASH	No FH
2	$Ser^{148}Ile$	5	No	ASH	FH
3	$Lys^{351}Glu$	12	Positive-negative	Mid-cavity	No FH
4	$Arg^{403}Gln$	13	Positive-neutral	ASH	SD, HCM and DCM in family
5	$Asn^{479}Ser$	15	No	ASH	No FH
6	$Glu^{500}Ala$	15	Negative-neutral	ASH	FH with much SD
7	$Gly^{571}Arg$	16	Neutral-positive	ASH	AF, No FH
8	$Arg^{663}His$	18	Positive-neutral	Mid-cavity	No FH, 'arrhythmia' in family
9	$Arg^{663}His$	18	Positive-neutral	Mid-cavity	PAF. SD and HCM in family
10	$Arg^{671}Cis$	18	Positive-neutral	ASH	PAF, no FH
11	$Ile^{736}Thr$	20	No	ASH	FH
12	$Ser^{782}Asn$	21	No	Mid-cavity	PAF, FH
13	$Val^{763}Gly$	21	No	ASH	DCM in family
14	$Met^{822}Leu$	22	No	ASH	PAF. FH and much AF in family
15	$Gln^{882}Glu$	22	No	ASH	FH
16	$Leu^{908}Val$	23	No	Mid-cavity	PAF, No FH
17	$Leu^{908}Val$	23	No	Mid-cavity	AF, SD in family
18	$Pro^{211}Leu^{*}$ + $Arg^{663}His$	7 & 18	No*	ASH	AF, AF in family
19	$Lys^{207}Gln$ homozygote	7	Positive-neutral	Apical	AF, heterozygotes bradycardic

ASH, Asymmetrical septal hypertrophy; SD, sudden cardiac death; AF, chronic atrial fibrillation; PAF, paroxysmal AF; FH, family history of HCM; DCM, dilated cardiomyopathy. The "No" refers to Pro211Leu.

TABLE 2. CLINICAL CHARACTERISTICS OF THE PATIENTS WITH AND WITHOUT *MYH7*-ASSOCIATED HCM

	All patients (n = *100*)	MYH7 *mutations* (n = *19*)	*Non-* MYH7 *HCM* (n = *81*)	p *value*
Age at diagnosis	35 ± 18	31 ± 17	36 ± 18	ns
Symptoms				
Asymptomatic	8	1 (5)	7 (9)	ns
Cardiac arrest	3	0	3 (4)	ns
Presyncope	66	15 (79)	51 (63)	ns
Syncope	33	11 (58)	22 (27)	0.01
Chest pain	72	14 (74)	58 (72)	ns
Dyspnea	86	16 (84)	70 (86)	ns
Palpitations	68	16 (84)	52 (64)	ns
CHF	13	5 (26)	8 (10)	0.07
Therapy				
Beta-blocker	51	7 (37)	44 (54)	ns
Calcium antagonist	42	8 (42)	32 (42)	ns
Pacemaker	37	5 (26)	32 (40)	ns
LVMM/PTSA	20	4 (21)	16 (20)	ns
Implantable defibrillator	8	3 (16)	5 (6)	ns
Atrial Fibrillation	32	9 (47)	23 (28)	0.17
Echocardiogram				
Septum (mm)	21 ± 7	21 ± 7	21 ± 7	ns
Posterior wall (mm)	11 ± 2	11 ± 2	10 ± 2	ns
LVIDd (mm)	45 ± 6	44 ± 7	46 ± 6	ns
LVIDs (mm)	26 ± 6	24 ± 5	27 ± 6	0.06
Fractional shortening (%)	42 ± 7	46 ± 7	41 ± 7	0.01
Left atrium (mm)	44 ± 10	52 ± 11	43 ± 9	<0.001
HCM Morphology				
ASH (± obstruction)	72	13 (68%)	69 (85%)	0.1
Apical	5	1 (5%)	4 (5%)	ns
Mid-ventricular	13	5 (28%)	8 (10%)	0.07
Exercise Test				
Duration (sec)	450 ± 199	438 ± 236	453 ± 194	ns
Abnormal BP response	23	5/11 (45%)	18/58 (31%)	ns

(), per cent; ns, not significant; LVMM, LV myotomy and myectomy; PTSA, percutaneous transluminal coronary septal ablation; LVIDd and LVIDs, LV internal dimension in diastole and systole; and BP, blood pressure.

spectively. The functinal domains of β-myosin's motor region affected by these mutations are shown in Figure 1.

Seventeen missense mutations were detected in 19 patients in 11 of the first 23 exons of the *MYH7* gene. Of these, 13 mutations, or 79%, are novel. Of the four previously described mutations, one ($Arg^{403}Gln$) is associated with a poor prognosis, one ($Leu^{908}Val$) is associated with a benign prognosis, and two (Ser^{782} Asn and $Arg^{663}His$) are associated with atrial fibrillation. Mutant alleles with the $Leu^{908}Val$ and $Arg^{663}His$ substitutions were found most commonly present in 2 and 3 patients, respectively. RFLP confirmed the presence of all novel mutations, except for $Asn^{479}Ser$ for which no enzyme was available.

Double heterozygosity and homozygosity: Both *MYH7* alleles were abnormal in two patients who have had severe forms of HCM. Patient 18 has double heterozygosity for the $Arg^{663}His$ and novel mutation $Pro^{211}Leu$ (Fig. 2A). He presented at the age of 56 years with fatigue and hypertension. An echocardiogram at the age of 63 years showed a markedly enlarged left atrium (61 mm), cardiac hypertrophy (septum 20 mm and posterior LV wall 10 mm), normal LV dimensions and systolic function (LV end-diastolic dimension, 36 mm; LV end-systolic dimension, 22 mm, fractional shortening, 39%), and LV outflow obstruction (estimated gradient, 55 mmHg). A further echocardiogram at the age of 76 years showed progression of the disease to LV wall thinning (septum 16 mm), impaired LV systolic function, and loss of outflow obstruction, associated with development of congestive cardiac failure and atrial fibrillation. He has been treated with diuretics, atrioventricular node ablation and pacemaker therapy. Of 3 siblings, one has both mutations, one has the $Arg^{663}His$ mutation only, and the third has neither (Fig. 2A). Both siblings with mutations were subsequently diagnosed with HCM complicated by atrial fibrillation, and the sibling with normal *MYH7* alleles has normal clinical findings, ECG, and echocardiogram.

Patient 19 is homozygous for a novel mutation $Lys^{207}Gln$ (Fig. 2B). HCM was diagnosed at the age of 47 years following an abnormal electrocardiogram. At the age of 64 years, an echocardiogram showed a markedly dilated left atrium (62 mm), apical HCM with basal septum measuring 15 mm increasing to 21 mm toward the apex, posterior LV wall 10 mm, LV end-diastolic dimension 52 mm, LV end-systolic dimension 30 mm, and no LV outflow gradient. The most recent echocardiogram, at the age of 74 years, shows increased left atrial (66 mm) and LV (56 mm in diastole) dimensions. He was treated with an implantable cardioverter-defibrillator following several episodes of syncope, and has had several therapeutic interven-

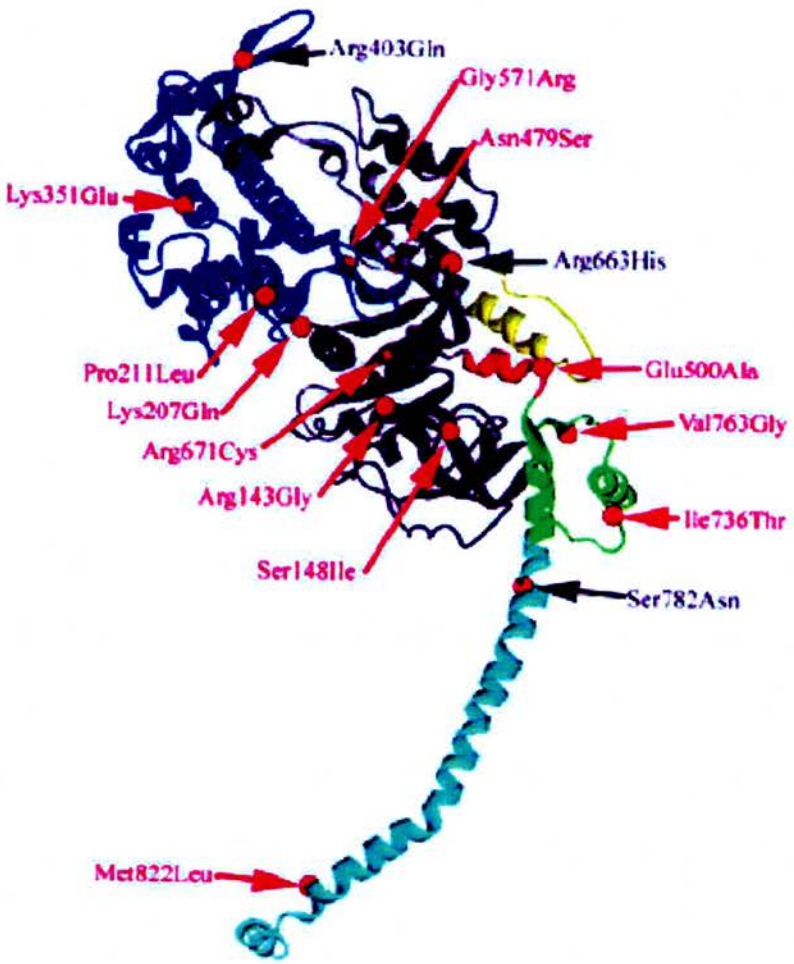

FIG. 1. The functional domain of β-myosin affected by each mutation was predicted from the crystalline structure of the highly homologous chicken fast skeletal myosin (PDB Id: 2MYS). This structure encompasses the motor region of myosin, which recent studies suggest comprises four major subdomains connected by three single-stranded joints; the amino-terminal subdomain (black); the upper 50-kDa subdomain (blue); the lower 50-kDa subdomain (gray), and the converter subdomain (green). Following the converter, a long helical segment of the heavy chain binds the essential light chain (ELC) and the regulatory light chain (RLC) together, forming the lever arm (Houdusse *et al.*, 2000). The motor region is shown with the positions of residues affected by each mutant marked; novel mutations are labeled in red. Approximate positions of residues Lys^{207}, Pro^{211}, and Gly^{571} are shown; residues Lys^{207} and Pro^{211} are contained in loop1 overlying the nucleotide pocket which is not resolved on the crystal structure. The region around Gly^{571} is also missing, and is in a part of the lower 50K subdomain thought to make contact with actin. Finally Gln^{882} and Leu^{908} are distal within the lever arm and are not seen on this model.

tions. He has also been admitted with congestive cardiac failure and is currently in functional class III and has chronic atrial fibrillation. His single surviving sibling, all 4 children, and three grandchildren are heterozygous for Lys^{207}Gln (Fig. 2D). This sibling, aged 80 years, was subsequently diagnosed with midventricular obstructive HCM and an interventricular septal wall thickness of 35 mm. The echocardiograms of a son (43 years), two daughters (46 and 40 years), and three grandchildren (3, 9, and 15 years) with the Lys^{207}Gln allele are normal. Several of the family members, including all three grandchildren with the Lys^{207}Gln allele but without cardiac hypertrophy, have a marked resting sinus bradycardia.

Comparison of clinical findings of MYH7 *and non-*MYH7*-associated HCM*: The two groups of HCM patients were similar with regard to age, gender, family history of HCM or sudden death, exercise performance, and need for pharmacological

FIG. 2. (**A**) Pherogram in patient 18 demonstrating double heterozygosity for Arg^{663}His and Pro^{211}Leu. In the pedigree, ages are in parentheses. One sibling has both *MYH7* mutations, another has Arg^{663}His only, and a third has neither. The siblings with mutations were subsequently diagnosed with HCM and atrial fibrillation. The sibling with normal *MYH7* alleles has a normal ECG and echocardiogram. (**B**) Pherogram demonstrating homozygosity for Lys^{207}Gln in patient 19. A sibling heterozygous for Lys^{207}Gln was diagnosed with midcavity HCM, but all other family members with a single mutant allele were normal.

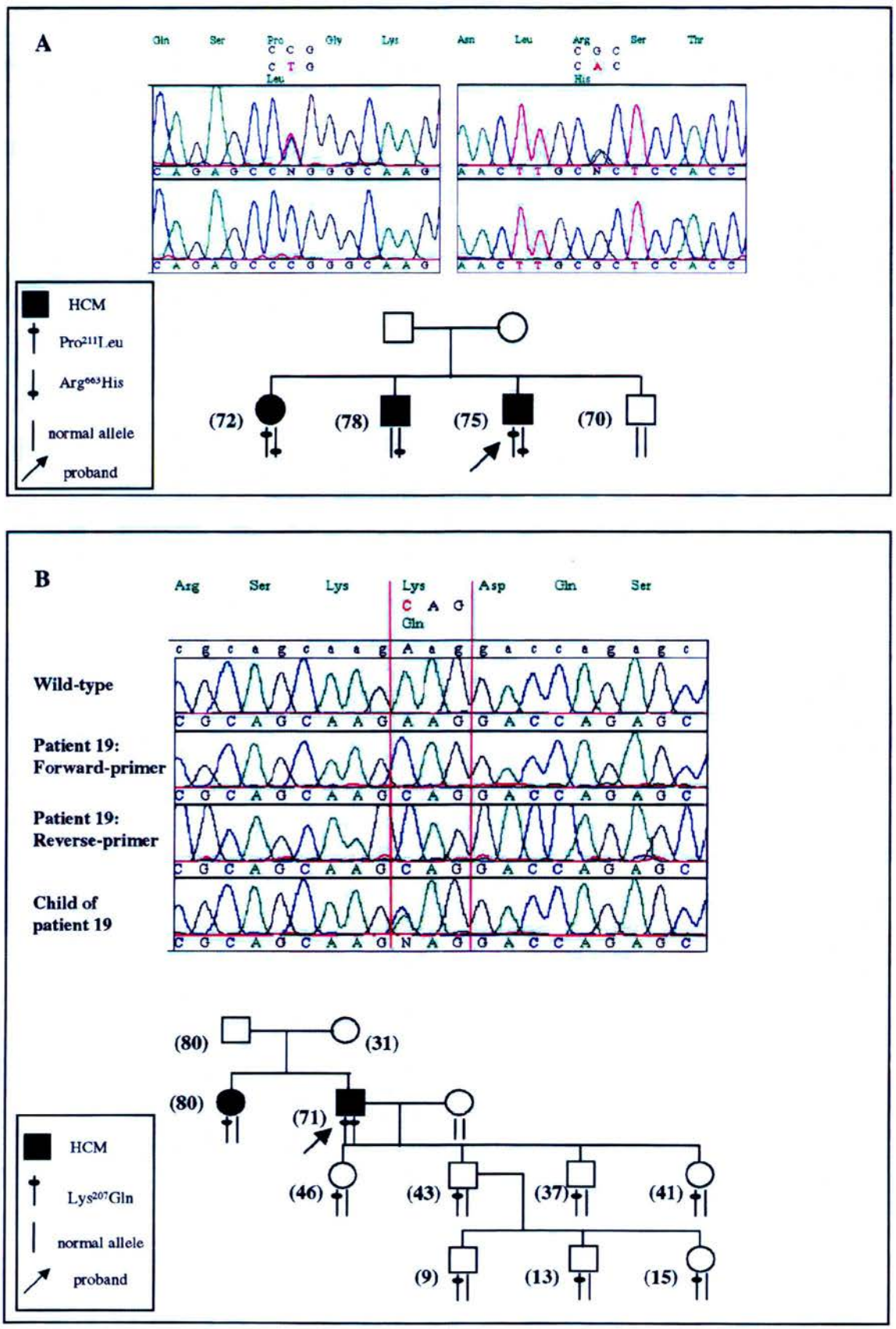
A
Gln Ser Pro Gly Lys Asn Leu Arg Ser Thr
Leu
His
HCM
Pro211Leu
Arg663His
normal allele
proband
(72) (78) (75) (70)
B
Arg Ser Lys Lys Asp Gln Ser
Gln
Wild-type
Patient 19: Forward-primer
Patient 19: Reverse-primer
Child of patient 19
(80) (31)
(80) (71)
(46) (43) (37) (41)
(9) (13) (15)
HCM
Lys207Gln
normal allele
proband

FIG. 2.

and/or other therapies. However, patients with *MYH7*-associated HCM were more likely to present with syncope and had greater left atrial dimensions, despite a similar severity of LV hypertrophy and higher LV fractional shortening (Table 2). Differences remain significant after exclusion of the 2 patients with double mutations. Patients with *MYH7*-associated HCM also had a greater tendency to develop atrial fibrillation, but this did not reach statistical significance.

Genetic analysis of normal controls

All novel mutations (except when a digest was unavailable) were absent in the RFLP analysis from the DNAs of 100 unrelated normal subjects (200 alleles). SSCP and direct sequencing of anomalous conformers for exons 5, 7, 12, 13, 15, 16, 18, 20, 21, 22, and 23 in 100 other unrelated normal individuals (200 alleles) identified several single-nucleotide polymorphisms that do not affect the amino acid encoded. (SSCP for exon 15 was performed on DNA of all 200 subjects, as RFLP was unavailable for $Asn^{479}Ser$). Thus, in 200 control alleles, no *MYH7* missense mutations were present in the 11 exons affected, and each novel mutation was absent from 400 control alleles.

DISCUSSION

Yield and utility of genetic screening methods

Our study demonstrates that *MYH7* mutations are detected in about 20% of patients with HCM. Most of these have not been described, indicating that mutations identified to date account for a fraction of all *MYH7*-associated HCM. Only 7 of 19 patients (4 of 17 mutations) had mutations that had been reported previously. Three of these ($Arg^{403}Gln$, $Arg^{663}His$ and $Leu^{908}Val$) are amongst a relatively few mutations with well-described natural histories that were similar to those of the unrelated patients in the present study (Epstein, 1992; Gruver *et al.*, 1999; Palmiter, 2000).

This degree of *MYH7* heterogeneity in HCM patients indicates that genetic screening must be able to identify novel mutations. As most mutations are rare, and may even be unique to certain families, genetic screening strategies relying on identification of previously determined mutations (such as RFLP) will have limited yields; clinically applicable techniques will most likely rely on sequencing-basedstrategies. Until automated high-throughput technologies, such as those based on chip arrays, become reliable, considerable time and expense are required for reliable mutation detection in HCM (Mohiddin *et al.*, 2001).

The validity of ascribing causative roles to *MYH7* mutations detected in individual HCM patients can be questioned. Pedigree expansion with linkage analysis, mutant protein functional assays, and transgenic models contribute to greater proof of causal relationships. In practice, definitive proof of causality will not be available for most detected mutations. The absence of a mutation from a number of control DNAs, identified by RFLP, is often cited as proof of cause. This study has shown that *MYH7* heterogeneity in HCM patients is such that most mutations will also be absent in a large population of unrelated HCM patients, just as in normal controls.

Although it has been assumed that *MYH7* sequence abnormalities are rare in the normal population, there has been little proof of this. We did not detect any missense sequence abnormalities by SSCP in 100 normal subjects in each of the exons identified as having mutations in this series of HCM patients, and did not find the specific abnormalities by RFLP in 100 other controls. Thus, *MYH7* is extremely conserved, and coding abnormalities are rare in the absence of cardiomyopathy. For future novel mutations found in the exons of this gene, it should not now be necessary to demonstrate their absence from control DNA as a routine element of the genetic diagnosis. For other genes, particularly myosin-binding protein C, this determination is yet to be made. Thus, these novel mutations are likely to cause HCM because (1) they involve highly conserved amino acids; (2) the mutations were absent in 400 alleles from normal subjects; (3) *MYH7* shows no sequence variation in 200 normal alleles and; and (4) HCM was present only in family members with the mutation.

A further complicating factor is that several sarcomeric gene mutations may be present in the same patient. Homozygosity and double heterozygosity for sarcomeric gene mutations have been described previously, but are assumed to be rare (Nishi *et al.*, 1994; Jeschke *et al.*, 1998; Richard *et al.*, 1999, 2000). In this series of consecutive patients, abnormalities in both alleles of *MYH7* were noted in 2% of the patients, or in 10% of those with *MYH7* mutations. If sufficiently frequent, occurrence of multiple mutations would confound linkage studies, particularly if heterozygosity involves other genes. It would also diminish our ability to attribute the disease to a particular mutation and may, in part, explain the phenotypic diversity characteristic of HCM. The likelihood of heterozygosity should also be considered in genetic counseling of at-risk family members.

Screening for molecular causes of HCM is only valuable for management of patients if the natural histories of the identified mutations are known and shared by families in which HCM is caused by the identical genetic defect. Unfortunately, the genetic diversity, the rarity of most mutations, and limited number of affected subjects, render phenotype–genotype correlations difficult. Therefore, this aspect of management for improving risk stratification in HCM is still very much in the clinical research domain.

Clinical differences between HCM caused by MYH7 *and non-*MYH7*-associated HCM*: Our findings suggest that *MYH7*-associated HCM differs from non-*MYH7*-associated HCM in certain important clinical respects. Despite better systolic function and similar LV wall hypertrophy and frequency of LV outflow obstruction, patients with *MYH7*-associated HCM had greater left atrial enlargement. This implies that *MYH7*-associated HCM is complicated by a greater degree of LV diastolic dysfunction. Affected patients were also more likely to present with syncope, perhaps related to an increased incidence of arrhythmias such as atrial fibrillation.

Implications for mutation–phenotype correlations: Our findings indicate that most HCM patients have an uncharacterized mutation whose rarity may disallow adequate description of the associated natural history in unrelated kindreds. Furthermore, once obtained, application of this information may be limited to few patients. The large number of *MYH7* mutations now described will permit testing of the hypothesis that the site and nature of mutations within the distinct functional domains of β-myosin determine clinical outcomes. A recent study linked a $Phe^{764}Leu$ mutation in *MYH7* to dilated cardiomyopathy. In our

study, a patient with obstructive HCM (septum of 32 mm) had a mutation in the preceding residue ($Val^{763}Gly$). However, a sibling with this mutation presented with cardiac failure and dilated cardiomyopathy as a teenager. In the two individuals with double-mutant alleles, one or both mutations occurred in a loop (loop 1) overlying the nucleotide-binding pocket. Thus, mutations in this region may, in isolation, result in only mild and poorly penetrant disease.

CONCLUSIONS

MYH7 mutations are found in one-fifth of HCM patients and are rare in normal subjects. Most HCM patients have mutations with undescribed natural histories. Mutation detection methods will have to rely on sequence-based methods. Compound heterozygosity occurs more frequently than has been appreciated and may be responsible for some of the phenotypic diversity and incomplete disease penetrance and for sporadic cases of HCM. Further work on genotype-functional consequence-phenotype correlations is needed and may be facilitated by grouping mutant proteins by functional domain affected.

A major advantage of a candidate gene-based screening method of consecutive unselected patients, such as in this study, is that patient selection is not biased toward those with a well-defined family history where the causative mutation is likelier to have a more penetrant and severe phenotype.

STUDY LIMITATIONS

Results of mutational analysis clearly depend on the referral patient population and the sensitivity of SSCP (about 85%). For simplicity, we analyzed only one gene. Analysis of other sarcomeric genes was beyond the scope of this investigation, but may demonstrate that multiple heterozygosity may be even more frequent and further complicate the phenotypic expression of HCM.

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IDENTIFICATION OF A GENE RESPONSIBLE FOR FAMILIAL WOLFF–PARKINSON–WHITE SYNDROME

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ABSTRACT

Background The Wolff–Parkinson–White syndrome, with a prevalence in Western countries of 1.5 to 3.1 per 1000 persons, causes considerable morbidity and may cause sudden death. We identified two families in which the Wolff–Parkinson–White syndrome segregated as an autosomal dominant disorder.

Methods We studied 70 members of the two families (57 in Family 1 and 13 in Family 2). The subjects underwent 12-lead electrocardiography and two-dimensional echocardiography. Genotyping mapped the gene responsible to 7q34–q36, a locus previously identified to be responsible for an inherited form of Wolff–Parkinson–White syndrome. Candidate genes were identified, sequenced, and analyzed in normal and affected family members to identify the disease-causing gene.

Results A total of 31 members (23 from Family 1 and 8 from Family 2) had the Wolff–Parkinson–White syndrome. Affected members of both families had ventricular preexcitation with conduction abnormalities and cardiac hypertrophy. The maximal combined two-point lod score was 9.82 at a distance of 5 cM from marker D7S636, which confirmed the linkage of the gene in both families to 7q34–q36. Haplotype analysis indicated that there were no alleles in common in the two families at this locus, suggesting that the two families do not have a common founder. We identified a missense mutation in the gene that encodes the γ2 regulatory subunit of AMP-activated protein kinase (*PRKAG2*). The mutation results in the substitution of glutamine for arginine at residue 302 in the protein.

Conclusions The identification of this genetic defect has important implications for elucidating the pathogenesis of ventricular preexcitation. Further understanding of how this molecular defect leads to supraventricular arrhythmias could influence the development of specific therapies for other forms of supraventricular arrhythmia. (N Engl J Med 2001;344: 1823-31.)

THE Wolff–Parkinson–White syndrome is the second most common cause of paroxysmal supraventricular tachycardia in most parts of the world and is the most common cause in China, being responsible for more than 70 percent of cases.[1] In Western countries, the prevalence of the Wolff–Parkinson–White syndrome is 1.5 to 3.1 per 1000 persons.[2-4] Tachycardias associated with the syndrome are usually paroxysmal and may produce symptoms of presyncope, syncope, and shortness of breath and cause sudden death. Conduction through an accessory pathway and the association of the Wolff–Parkinson–White syndrome with supraventricular tachycardia have led to the creation of an in vivo reentry model for arrhythmias.[5] Research on the Wolff–Parkinson–White syndrome has appropriately focused on the atrioventricular pathways, which led to ablation as an effective therapy.[6] We evaluated two families with familial Wolff–Parkinson–White syndrome in which the probands presented with syncope and the electrocardiographic features of the syndrome. A clinical evaluation of members of both families was followed by linkage analysis to identify the chromosomal location of the causative gene.

METHODS

Clinical Evaluation

Written informed consent was obtained from all participants according to the guidelines of Baylor College of Medicine; the National Heart, Lung, and Blood Institute of the National Institutes of Health; and the University of Ottawa Heart Institute. Subjects were evaluated by means of a detailed analysis of their medical history, a physical examination, 12-lead electrocardiography, and two-dimensional echocardiography. A total of 57 members of Family 1 and 13 members of Family 2 were examined. Two subjects in Family 1 and six subjects in Family 2 underwent invasive electrophysiologic study.

Ventricular preexcitation was diagnosed on the basis of the presence of a short PR interval (<120 msec) with a widened QRS complex (>110 msec) or an abnormal initial QRS vector (a delta wave). In the case of subjects who had a pacemaker, base-line 12-lead electrocardiograms were obtained from their medical records, when possible. Sinoatrial abnormalities and conduction disease were diagnosed if chronotropic incompetence or high-grade sinoatrial or atrioventricular block was present on the electrocardiogram. Left ventricular hypertrophy was diagnosed if the thickness of the septal wall or the left ventricular free wall was at least 13 mm.

Chromosomal Mapping and Identification of Candidate Genes

Peripheral blood was obtained from each family member we evaluated. DNA was extracted from white cells, and lymphocytes

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were isolated for the development of transformed cell lines.[7] Genotyping was carried out with short tandem-repeat polymorphisms from the 7q34–q36 region. We examined a total of 12 markers from the Genethon linkage map, and 2 additional polymorphic repeats were identified from the published sequence of P1-derived artificial chromosomes in the region. An autosomal dominant pattern of inheritance was assumed, and penetrance was estimated to be 99 percent on the basis of the observed pattern of inheritance. The frequencies of the disease allele and the normal allele were assumed to be 0.0001 and 0.9999, respectively. The allele frequencies in the case of markers were calculated to be 1/n, where n is the number of alleles observed in the two pedigrees. Two-point linkage analysis was performed with version 5.2 of the linkage program.[8] Once the disease-causing locus was identified, we used a candidate-gene approach to identify the responsible gene.

We identified sequences of P1-derived artificial chromosomes from the Human Genome Project by searching draft sequences containing Genethon markers mapped to the region. We identified two additional informative polymorphic markers from these sequences to narrow the critical region. We then entered each sequence into the National Center for Biotechnology Information BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST/) to identify candidate genes, one of which was the gene that encodes the γ2 subunit of AMP-activated protein kinase (*PRKAG2*).

Identification of the Mutation in the *PRKAG2* Gene

Exon–intron boundaries for protein-encoding sequences of *PRKAG2* (GenBank accession number, AJ249976) complementary DNA (cDNA) were identified in the GenBank data base with use of the BLAST search program within the following P1-derived artificial chromosome clones: RP11-79612 (cDNA bp 1 to 205; GenBank accession number, AC074257), RP5-1127D14 (bp 557 to 843; GenBank accession number, AC006358), and RP4-563H24 (bp 844 to 1800; GenBank accession number, AC006966). Intronic primers were derived on the basis of these sequences. Primer sequences are available with the full text of this article at http://www.nejm.org and at http://www.bcmcardiofellows.org. Fragments of genomic DNA were amplified by the polymerase chain reaction (PCR), and the products were purified with use of the QIAquik PCR purification kit (Qiagen, Valencia, Calif.). In the case of protein-encoding sequences (bp 206 to 556) that were not identified in the GenBank data base, RNA was isolated from lymphoblastoid cells with a random primer and reverse-transcribed with use of the Prostar system (Stratagene, Cedar Creek, Tex.). Direct sequencing reactions were performed in both the sense and antisense directions on an automated sequencer (Prism 377, Perkin–Elmer Applied Biosystems, Foster City, Calif.) with use of a technique involving dye-labeled terminators.[9]

RESULTS

Clinical Evaluation

Analysis of Families 1 and 2 (Fig. 1) showed that the mode of transmission of the Wolff–Parkinson–White syndrome was consistent with an autosomal dominant pattern of inheritance. Transmission occurred with high penetrance but with a variable degree of expression. Initial clinical presentations included reports of palpitations, presyncope, and syncope. The onset of clinical symptoms typically occurred in late adolescence or the third decade of life. All 24 subjects (16 from Family 1 and 8 from Family 2) for whom base-line 12-lead electrocardiograms were available had evidence of ventricular preexcitation. Paroxysmal atrial fibrillation or flutter occurred in association with the Wolff–Parkinson–White syndrome in 44 percent of the subjects in Family 1 and 38 percent of the subjects in Family 2. Twelve members of Family 1 had had recurrent syncope. A total of eight subjects underwent invasive electrophysiologic studies, and a total of 10 anomalous conduction pathways were identified. Two of the eight (Subjects III-5 and IV-1 in Family 2) had two accessory connections, or tracts. Typical accessory pathways, including two right-sided pathways and one posterolateral pathway, were identified. Five of the eight family members who were studied had evidence of preexcitation with decremental conduction properties.

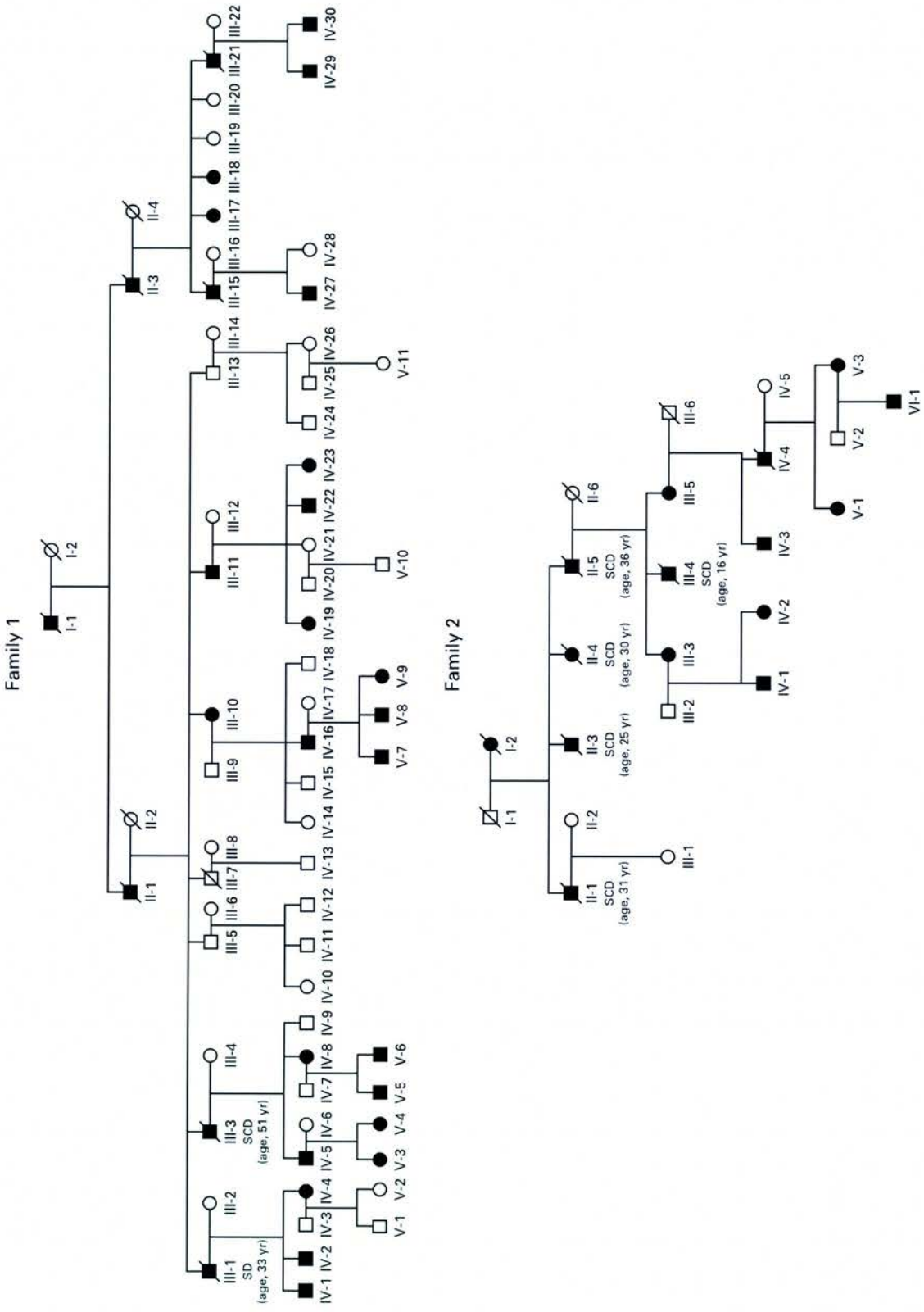
In addition to preexcitation, other forms of conduction disease were seen. Three young women (Subjects V-3 and V-4 in Family 1 and Subject V-1 in Family 2) had resting heart rates of less than 50 beats per minute and an inadequate heart-rate response to exercise. Progression to high-grade sinoatrial or atrioventricular block requiring the implantation of a pacemaker occurred in 76 percent of the affected members of both families who were older than 30 years of age. Cardiac hypertrophy was identified in 8 of 31 affected subjects (26 percent) who were evaluated. In two members of Family 1 (Subjects IV-1 and IV-16) hypertrophy progressed to left ventricular dysfunction (ejection fraction, <40 percent). In one member of Family 2 who had left ventricular hypertrophy (Subject IV-4) severe left ventricular dysfunction developed that required cardiac transplantation at the age of 42 years. Six patients died before the age of 40 years, but whether they had the Wolff–Parkinson–White syndrome or other features of the phenotype is unknown, since neither medical records nor postmortem findings were available.

Chromosomal Location and Haplotype Analysis

In determining the chromosomal location of the gene responsible for familial Wolff–Parkinson–White syndrome in Family 1, we first assessed whether there was linkage to 7q34–q36, a locus previously identified as the site of a gene responsible for a familial form of hypertrophic cardiomyopathy and the Wolff–Parkinson–White syndrome.[10] The maximal two-point lod score was 9.82 for marker D7S636 at a distance of 0 cM. The maximal two-point lod score was 1.64

Figure 1 (facing page). Pedigrees of Two Families with Familial Wolff–Parkinson–White Syndrome.

The members of each family who died suddenly of an undetermined cause (SD) or from cardiac causes (SCD) and the age at death are shown. Solid symbols denote affected family members, circles female family members, squares male family members, and symbols with a slash deceased family members.


Family 1
I-1
I-2
II-1
II-2
II-3
II-4
III-1
SD
(age, 33 yr)
III-2
III-3
SCD
(age, 51 yr)
III-4
III-5
III-6
III-7
III-8
III-9
III-10
III-11
III-12
III-13
III-14
III-15
III-16
III-17
III-18
III-19
III-20
III-21
III-22
IV-1
IV-2
IV-3
IV-4
IV-5
IV-6
IV-7
IV-8
IV-9
IV-10
IV-11
IV-12
IV-13
IV-14
IV-15
IV-16
IV-17
IV-18
IV-19
IV-20
IV-21
IV-22
IV-23
IV-24
IV-25
IV-26
IV-27
IV-28
IV-29
IV-30
V-1
V-2
V-3
V-4
V-5
V-6
V-7
V-8
V-9
V-10
V-11
Family 2
I-1
I-2
II-1
SCD
(age, 31 yr)
II-2
II-3
SCD
(age, 25 yr)
II-4
SCD
(age, 30 yr)
II-5
SCD
(age, 36 yr)
II-6
III-1
III-2
III-3
III-4
SCD
(age, 16 yr)
III-5
III-6
IV-1
IV-2
IV-3
IV-4
IV-5
V-1
V-2
V-3
VI-1

for marker D7S2439 in the analysis involving Family 2. The maximal combined lod score for both families was 9.82 at a distance of 5 cM from marker D7S636. Combined haplotype analysis indicated a shared region among affected members flanked by markers D7S2461 and D7S483 corresponding to a genetic distance of less than 2.6 cM. Haplotype analysis with the use of a total of 10 polymorphic-repeat markers and 1 single-nucleotide polymorphism for Family 1 and Family 2 indicated that there were no alleles in common segregating at this locus, suggesting the two families do not share a recent common

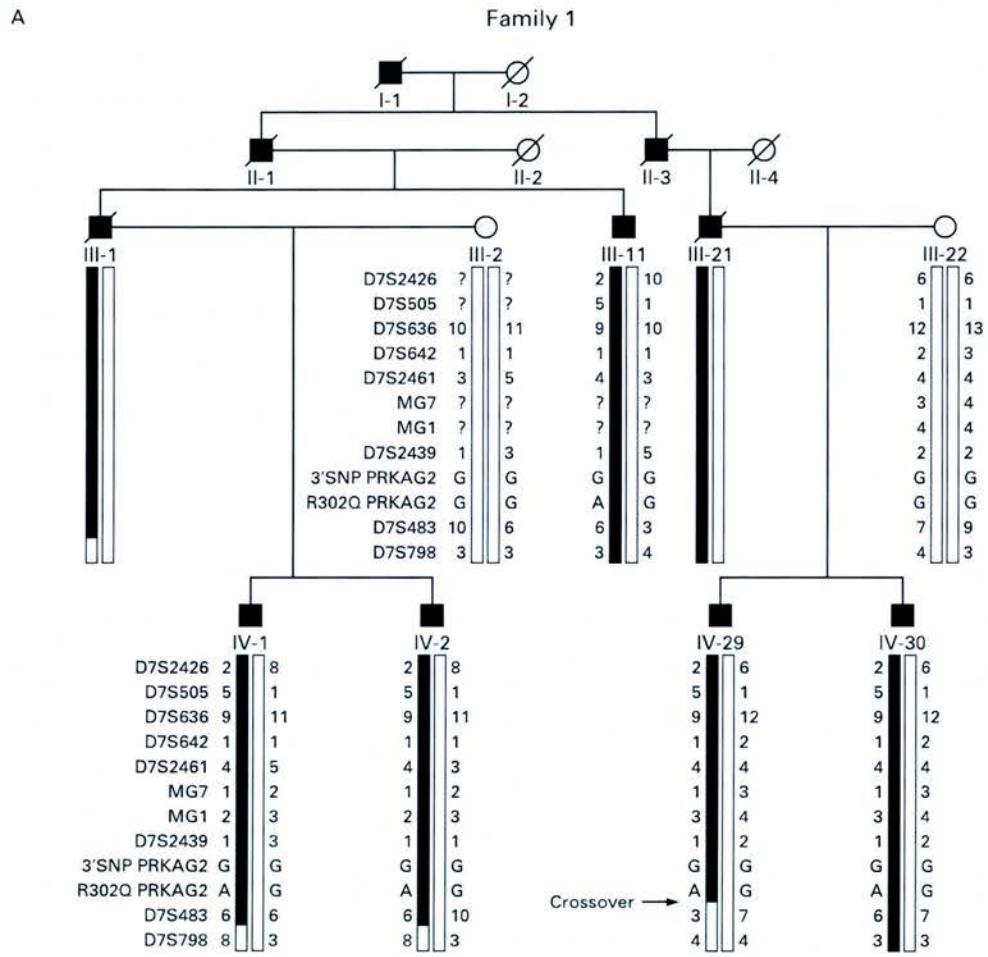
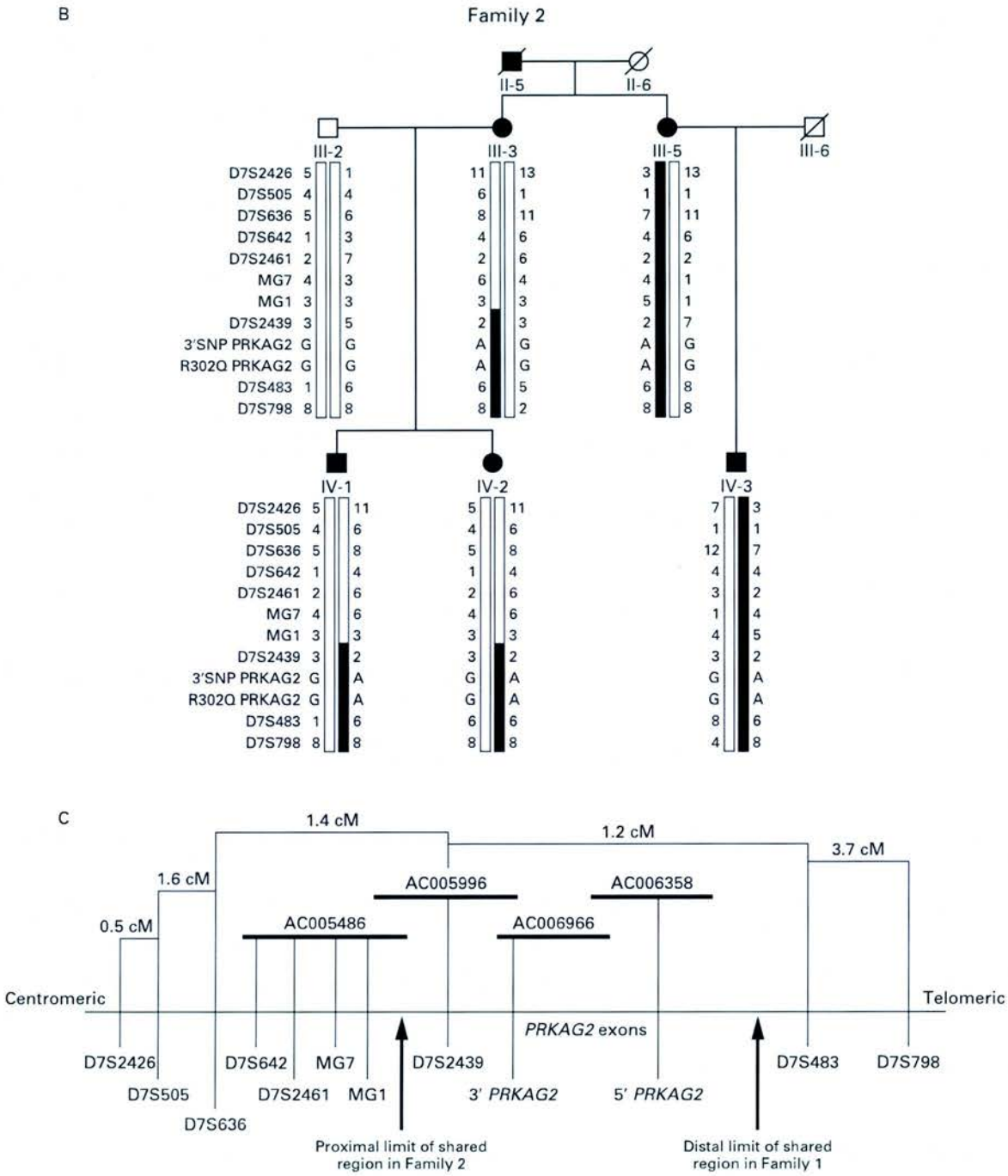


Figure 2. Haplotypes, Recombination, and Genetic Map of the *PRKAG2* Genomic Region in Family 1 and Family 2.

Panel A shows the results of haplotype analysis of nine members of Family 1 involving numerous polymorphic-repeat markers and one single-nucleotide polymorphism in the 3′ untranslated region (3′ SNP) of *PRKAG2*. MG7 and MG1 are novel markers identified on the basis of the sequence of P1-derived artificial chromosomes. In Subject IV-29, a crossover occurred between D7S2439 and D7S483, marking the distal limit of the shared region in this family. In Panel B, the results of haplotype analysis of six members of Family 2 involving the same polymorphic markers indicate that a crossover occurred between D7S2439 and MG1 in Subject III-3, marking the proximal limit of the shared region in this family. All affected members of both families had the R302Q mutation in *PRKAG2*. All affected members of Family 2 had a mutation (the substitution of adenine for guanine) at bp 1912 in the 3′ untranslated region of *PRKAG2*, whereas none of the affected members of Family 1 had this mutation. Panel C shows the distance (in centimorgans) of each marker shown in Panels A and B from the region of interest. Black bars in Panels A and B show the region shared by all the affected members of both families. In Panel C, an integrated map of the *PRKAG2* genomic region shows the genetic distances in centimorgans derived from the Genethon linkage map and the approximate positions of the crossovers (arrows). The drawing is not to scale. The accession numbers of the sequenced P1-derived artificial chromosomes are shown above the bold lines.



founder. The distinct haplotype of affected members in each family is shown in Figure 2.

Identification of the Mutation in the *PRKAG2* Gene

After we determined that *PRKAG2* was in the critical genomic region, we amplified and sequenced from genomic DNA affected and unaffected family members. A sequence variation (the substitution of adenine for guanine) was identified that corresponded to bp 995 of the *PRKAG2* cDNA sequence in all affected members in Family 1. This change results in a change in the amino acid at residue 302 from ar-

ginine to glutamine (R302Q). The identical sequence variation was subsequently found in all affected members of Family 2. Unaffected relatives in both families had no evidence of this sequence variation. The same PCR product from genomic DNA was sequenced in 300 chromosomes from control subjects selected from the general population, which showed no evidence of sequence variation. The existence of the R302Q mutation was independently confirmed by analysis of the results of restriction-enzyme digestion (Fig. 3). Taken together, the findings indicate that this mutation in *PRKAG2* is likely to cause familial Wolff–Parkinson–White syndrome. An additional sequence variation (the substitution of adenine for guanine) was identified at bp 1912 in the 3′ untranslated region of *PRKAG2* that was present in all affected members of Family 2, but not Family 1. This finding further confirms that these two families are unrelated. We have analyzed the protein-encoding sequence of *PRKAG2* in genomic DNA from five patients with sporadic cases of the Wolff–Parkinson–White syndrome and did not detect any mutations.

DISCUSSION

We studied two families in which the probands presented with the Wolff–Parkinson–White syndrome. Of the total of 70 family members whom we examined, 31 were affected in five generations. The trait is inherited in an autosomal dominant pattern with complete penetrance and variable degrees of expression. All affected subjects had electrocardiographic evidence of preexcitation. Certain features occurred more commonly in our subjects than in those with sporadic Wolff–Parkinson–White syndrome. Paroxysmal atrial fibrillation and flutter were present in 44 percent of the subjects in Family 1 and 38 percent of the subjects in Family 2, an incidence that is significantly higher than the incidence of 15 to 20 percent that has been reported for sporadic Wolff–Parkinson–White syndrome.[11,12] In addition, conduction abnormalities and cardiac hypertrophy are uncommonly associated with sporadic Wolff–Parkinson–White syndrome but were commonly seen in the two families that we studied.[13] Electrophysiological studies showed a higher than expected incidence of preexcitation with decremental conduction properties.[14,15] Six subjects died suddenly from cardiac causes before the age of 40 years. Although definitive diagnoses could not be confirmed in these obligate gene carriers, this finding suggests that the risk of sudden death is higher in patients with familial Wolff–Parkinson–White syndrome than in patients with sporadic cases. Nevertheless, the Wolff–Parkinson–White syndrome is a well-recognized cause of sudden death,

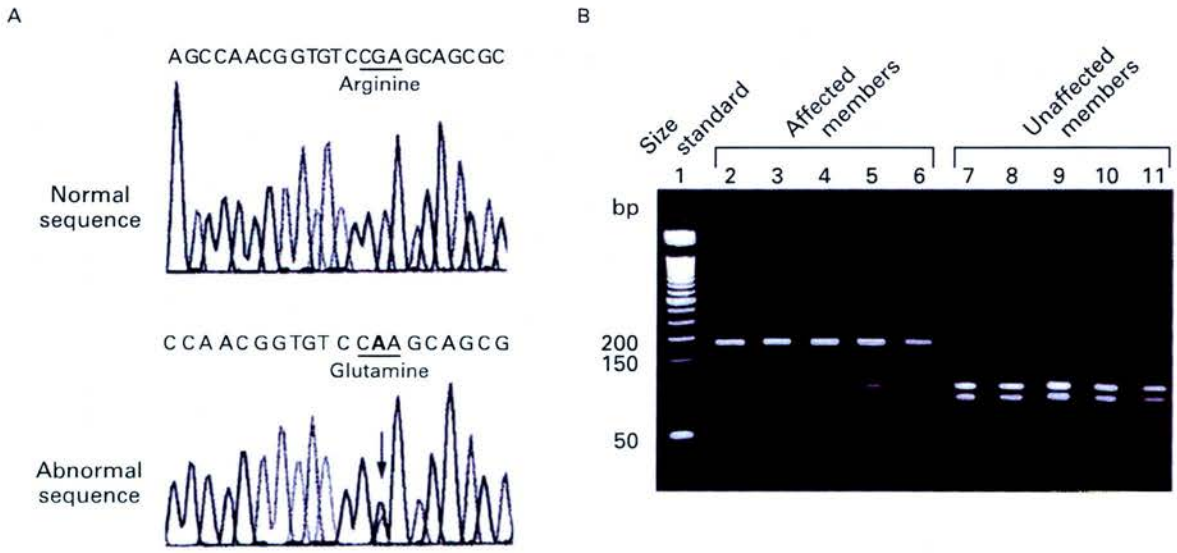


Figure 3. Sequence Analysis and Secondary Confirmation of the *PRKAG2* Mutation.

Sequence analysis of the sense strand of genomic DNA from an affected member of Family 1 indicates the substitution of adenine (A) for guanine (G) at bp 995 of PRKAG2 complementary DNA. This results in the substitution of glutamine for arginine (R302Q) in the PRKAG2 protein (arrow in Panel A). The mutation contained within a 190-bp amplicon abolishes an *Hpy*188I restriction-enzyme site, resulting in the persistence of this fragment in affected members after restriction-enzyme digestion (Panel B).

and in studies of young survivors of sudden cardiac arrest who did not have gross structural heart disease, the syndrome was present in up to 33 percent of patients.[16,17]

In both families the gene responsible mapped to 7q34–q36, which had been previously documented to be the locus responsible for disease in a family with combined hypertrophic cardiomyopathy and the Wolff–Parkinson–White syndrome.[10] Using the candidate-gene approach, we identified the gene *PRKAG2*, which encodes the γ2 regulatory subunit of AMP-activated protein kinase. The genetic defect is a point mutation resulting in the substitution of glutamine for arginine (R302Q). That the mutation was responsible for the phenotype in these families was confirmed by the following findings: the mutation was present in all affected members of two unrelated families, the mutation was absent in all unaffected members of both families as well as in 300 chromosomes from control subjects, and the substituted ar-

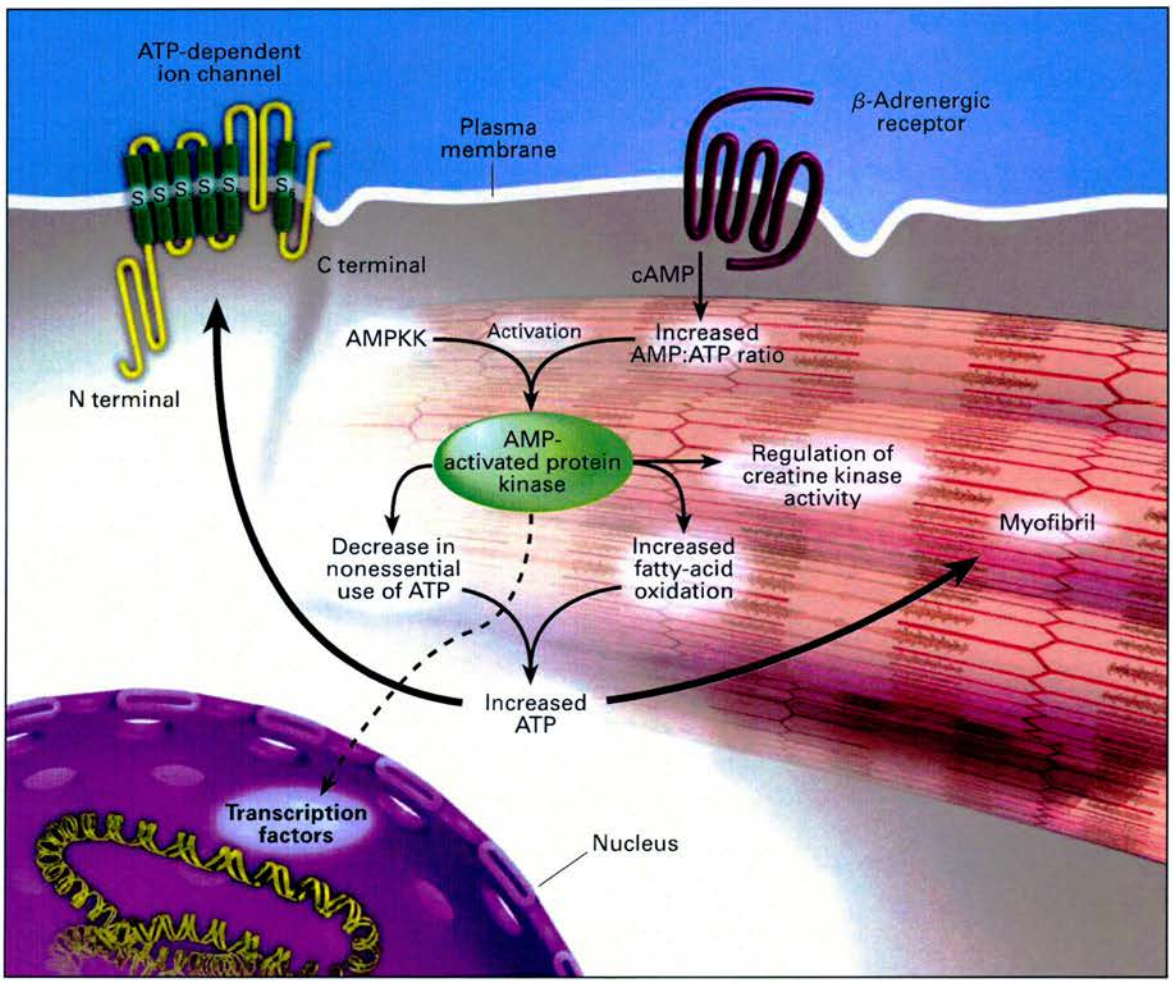


Figure 4. Regulation and Function of AMP-Activated Protein Kinase.

In response to an elevated ratio of AMP to ATP, AMP-activated protein kinase is activated by both direct AMP binding and phosphorylation by AMP-activated protein kinase kinase (AMPKK). AMP-activated protein kinase may also be activated in response to the increase in cyclic AMP (cAMP) and resultant increase in the AMP:ATP ratio induced by β-adrenergic stimulation. The diverse functions of AMP-activated protein kinase include the inactivation of nonessential ATP-consuming pathways, the regulation of the activity of creatine kinase, and the restoration of ATP levels through increased fatty-acid oxidation to meet vital cellular needs such as ion-channel activity and sarcomeric contraction. In addition, AMP-activated protein kinase may migrate to the nucleus and regulate gene transcription.

ginine is highly conserved across several species, reflecting the functional biologic importance of this amino acid.

The same mutation occurred in the two families despite the absence of a common founder. The absence of a common founder is based on the following evidence: the two families were not known to be related, they did not share alleles segregating in the vicinity of the *PRKAG2* gene, and the single-nucleotide polymorphism identified at bp 1912 of *PRKAG2* was present in all affected members of Family 2, but not Family 1. Nucleotide substitutions tend not to occur at random. Substitutions of adenine for guanine, as occurred in the mutation identified in the two families we studied, are 10 to 40 times as frequent as other base substitutions.[18] The substitution in *PRKAG2* occurred at a CG doublet, and these doublets are often referred to as "hot spots" for mutation.[19]

The *PRKAG2* gene consists of 569 amino acids with a calculated molecular mass of 63 kd.[20] The γ subunit of the AMP-activated protein kinase heterotrimer functions as the AMP-binding site, thus regulating the activity of the protein. However, it is not possible to determine whether the R302Q mutation directly affects AMP binding, since the sequence of the AMP-binding site is not known.[21]

AMP-activated protein kinase functions as a metabolic sensor in cells, responding to cellular energy demands by regulating diverse ATP-using pathways and ATP-generating pathways.[22] In the presence of an elevated ratio of AMP to ATP, AMP-activated protein kinase is activated by the direct binding of AMP, which exposes a threonine of the catalytic unit. The threonine is then phosphorylated by an upstream kinase (AMP-activated protein kinase kinase).[20] Activation decreases the use of ATP for nonessential functions and stimulates ATP-generating pathways, conserving ATP for more vital cellular requirements (Fig. 4). The PRKAG2 isoform has a high level of expression in cardiac tissue, and it is also present in skeletal muscle, the brain, the placenta, the liver, the kidneys, and the pancreas.[20]

The Wolff–Parkinson–White syndrome is thought to be due to accessory pathways derived from muscle fibers that provided direct continuity between atrial and ventricular myocardium during cardiogenesis.[23] The molecular defect that we found may in some way inhibit the normal regression of muscle fibers during atrioventricular septation. Although the propensity for arrhythmias in patients with familial Wolff–Parkinson–White syndrome is well known, the mechanism that triggers these episodes at the molecular level is not understood. The activation of AMP-activated protein kinase in response to β-adrenergic stimulation could account for the development of tachyarrhythmias during exercise or metabolic stress.[24,25]

It is unclear whether the R302Q mutation acts as an activating or inactivating mutation. However, the mutation probably leads to an alteration in the phosphorylation of downstream substrates within the heart. Potential targets are likely to include enzymes involved in energy metabolism and ion-channel proteins. An important task will be to identify the proteins whose phosphorylation is affected by this mutation. Although AMP-activated protein kinase affects gene expression,[26,27] its role during cardiac development is unknown. The identification of this genetic defect has important implications for the diagnosis and treatment of the Wolff–Parkinson–White syndrome. The relation between the role of AMP-activated protein kinase in cardiac electrophysiology and the management of supraventricular arrhythmias and conduction disease remains to be elucidated.

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Pediatric Cardiology

Myocardial Bridging Does Not Predict Sudden Death in Children With Hypertrophic Cardiomyopathy but Is Associated With More Severe Cardiac Disease

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OBJECTIVES We sought to examine the association between systolic compression of sections of epicardial coronary vessels (myocardial bridging) with myocardial perfusion abnormalities and clinical outcome in children with hypertrophic cardiomyopathy (HCM).

BACKGROUND It has recently been suggested that myocardial bridging is an important cause of myocardial ischemia and sudden death in children with HCM.

METHODS Angiograms from 57 children with HCM were reviewed for the presence of bridging (50% or more maximum systolic arterial compression). QT interval indices, echocardiographic and cardiac catheterization findings, treadmill exercise tests, exercise thallium scintigraphy, Holter monitoring and electrophysiologic study findings were compared in children with and without bridging. The findings were also related to the presence or absence of compression of septal branches of the left anterior descending artery (LAD).

RESULTS Bridging was present in 23 (40%) of the children. Multiple coronary arteries were involved in four children. Bridging involved the LAD in 16 of 28 (57%) affected vessels. Myocardial perfusion abnormalities were present in 14 of 30 (47%) children without bridging and in 17 of 22 (94%) children with bridging, $p = 0.002$. However, bridging was associated with more severe septal hypertrophy (19 ± 8 mm vs. 28 ± 8 mm, $p < 0.001$), a higher septum:posterior wall thickness ratio (2.7 ± 1.2 vs. 1.8 ± 0.9, $p < 0.001$), and higher left ventricle (LV) outflow gradient (45 ± 37 mm Hg vs. 16 ± 28 mm Hg, $p = 0.002$). Compression of septal LAD branches was present in 37 (65%) of the children and was significantly associated with bridging, severity of LV hypertrophy and outflow obstruction. Multivariate analysis demonstrated that LV septal thickness and septal branch compression, and not bridging, were independent predictors of thallium perfusion abnormalities. There was a 90% power at 5% significance to detect an effect of bridging on thallium abnormalities at an odds ratio of 3. Bridging was also not associated with significantly greater symptoms, increased QT and QTc intervals and QTc dispersion, ventricular tachycardia on Holter or induced at EP study, or a worse prognosis.

CONCLUSIONS Bridging and compression of septal branches of the LAD are common in HCM children and are related to magnitude of LV hypertrophy. Left ventricular hypertrophy and compression of intramyocardial branches of the epicardial coronary arteries may contribute to myocardial perfusion abnormalities. Our findings suggest that bridging does not result in myocardial ischemia and may not cause arrhythmias or sudden death in HCM children. (J Am Coll Cardiol 2000;36:2270–8) © 2000 by the American College of Cardiology

Chest pain and myocardial ischemia are common in hypertrophic cardiomyopathy (HCM). About two-thirds of adult patients have regional myocardial perfusion abnormalities as demonstrated by exercise thallium-201 scintigraphy and positron emission tomography studies (1,2). Further evidence for myocardial ischemia has been provided by stress-induced anaerobic metabolism with reduced myocardial lactate consumption or lactate production in patients with a history of chest pain (1). Children with HCM are at higher risk for sudden death than adult patients, and exercise thallium myocardial perfusion abnormalities occur more frequently in children who present with syncope or cardiac arrest (3).

Several mechanisms have been proposed to explain myocardial ischemia in HCM. These include increased metabolic demand (4,5), dysfunction and paucity of the coronary microvasculature (5–8), elevated left ventricle (LV) diastolic pressure reduced vasodilator reserve (9,10), bridging of epicardial coronary arteries (1,11–13) and compression of septal branches of the left anterior descending artery (LAD) (4,14–17).

The significance of compression of sections of epicardial coronary vessels has been controversial. In a recent communication, Yetman et al. (13) report that bridging is an important cause of angina and myocardial ischemia, is associated with greater dispersion of the QT interval and is a significant risk factor for ventricular arrhythmias and sudden death in children

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Abbreviations and Acronyms

ECG = electrocardiogram
EP = electrophysiologic study
HCM = hypertrophic cardiomyopathy
LAD = left anterior descending artery
LV = left ventricle
QT = QT interval
QTc = corrected QT interval
VT = ventricular tachycardia

with HCM. Given the potential therapeutic implications of these observations, we have reviewed the prevalence and clinical correlates of bridging in HCM children evaluated at the National Institutes of Health.

METHODS

Patients. The study comprised children with HCM who underwent coronary angiography at the National Institutes of Health between January 1989 and April 1999 and included children whose records were assessed retrospectively. All children underwent M-mode and 2D echocardiography and HCM was defined as a hypertrophied, nondilated LV in the absence of another cause for the increased cardiac mass. Studies were performed after >5 half-lives off all cardiac medications.

Measurements. HEMODYNAMIC STUDIES. Cardiac catheterization was performed using intravenous sedation. Right heart pressures and cardiac output were measured with a thermodilution Swan–Ganz catheter. Left ventricle outflow pressure gradient was recorded from the side-arm of a femoral artery sheath, a 5F or 6F end-hole pigtail catheter placed in the LV and also by careful withdrawal of the catheter from LV apex to ascending aorta. Significant obstruction was defined as a subvalvular gradient of >30 mm Hg at rest or >50 mm Hg following provocation.

ANGIOGRAPHIC ASSESSMENT OF BRIDGING. Selective coronary angiography was performed using standard Judkins catheter technique. Cineangiographic films were reviewed independently by two observers, one of whom was blinded to clinical histories. All coronary segments showing evidence of bridging were assessed quantitatively. A 35 mm cineprojector incorporating a digital video camera was used to capture images of each bridged artery. Angiographic boundaries were detected automatically by the analysis software and manual corrections made when necessary. Absolute arterial dimensions (mm) were measured using the coronary artery catheter as reference. The following were estimated: length of bridged segment and severity of bridging [(systolic diameter of the artery just distal to bridging minus systolic diameter of the bridged segment) ÷ systolic diameter of the artery just distal to bridging × 100]. Coronary bridging was defined as a maximum systolic compression ≥50% (13).

SYSTOLIC COMPRESSION OF SEPTAL BRANCHES OF THE LAD. Septal perforator compression was defined as the transient occlusion of septal branches of the LAD during systole and was determined to be either present or absent by two independent observers.

EXERCISE DURATION, HEART RATE AND BLOOD PRESSURE RESPONSES TO EXERCISE. Treadmill exercise tests were performed using the Bruce protocol. Exercise duration, heart rate and blood pressure responses were recorded. An abnormal exercise blood pressure was defined as <20 mm Hg increase in peak sphygmomanometer systolic pressure compared with resting value (18).

MYOCARDIAL ISCHEMIA. Exercise thallium scintigraphy was performed following an overnight fast by previously described methods (3). At peak exercise, up to 2 mCi of thallium (dose determined by patient weight) was administered intravenously and the patient exercised for an additional 45 to 60 s, with thallium images obtained within 10 min after exercise. Repeat images were acquired approximately 3 to 4 h later following administration of up to 1 mCi of thallium. Horizontal long axis, vertical long axis, and short axis tomograms were reconstructed and analyzed qualitatively in the anterior, apical, inferior, septal and lateral regions. Stress and reinjection images were normalized to the region with the maximum myocardial activity on the stress images and each region was assigned as abnormal or normal. A region of reduced thallium uptake was determined to be abnormal but reversible if it normalized on reinjection images, and to be abnormal and irreversible if it persisted. The presence or absence of apparent cavity dilation was also determined.

ARRHYTHMIAS. Holter recordings obtained nearest the time of angiography were analyzed for the presence or absence of ventricular tachycardia (VT), defined as >3 beats duration. The QT and QTc intervals were measured from the 12-lead electrocardiogram (ECG) by a physician blinded to the clinical findings. QTc dispersion was defined as the difference between the longest and shortest QTc intervals measured on all 12-lead ECG leads. Electrophysiologic (EP) studies were performed at the time of the cardiac catheterization in 36 of the children using previously described methods (19).

Statistical analysis. Patient data are presented as mean ± 1 SD. Two-sample data were compared by Student *t* test. Cardiac survival rates for patients with and without bridging were determined by the Kaplan–Meier estimates and compared with the logrank test. Cardiac events were defined as sudden death or resuscitated cardiac arrest. Multivariate logistic regression analysis was used to determine the independent contributions of LV wall thickness, LV outflow obstruction, septal compression and bridging to presence of thallium perfusion abnormalities. The power of the study to detect a contribution of bridging to thallium abnormalities was calculated on the binomial model with the logit link with and without adjustment for confounding effects of the other vari-

Table 1. Clinical Findings in the HCM Children: Prevalence of Risk Factors

Cardiorespiratory arrest, syncope and/or presyncope	35 (61%)
Family history of ≥2 premature sudden death	12 (21%)
Myocardial ischemia	31 (54%)
VT during ambulatory Holter monitoring	14 (25%)
Genetic abnormality associated with a poor prognosis	4 (6%)
Severe LV hypertrophy (LV wall thickness ≥30 mm)	12 (21%)
Flat* or hypotensive BP response to baseline exercise	26 (46%)
≥1 clinical features associated with sudden death	53 (93%)

*<20 mm Hg increase in systolic arterial blood pressure (BP).

ables (20). A p value < 0.05 was considered statistically significant.

RESULTS

Characteristics of the study population. From a total of 163 children admitted for assessment during this 10-year period, 57 (35%) had selective coronary angiograms of sufficient quality for quantitative analysis. Of these, 35 (61%) were catheterized before the identification of coronary bridging as a potential determinant of outcome and their angiograms and case histories were reviewed retrospectively. Indications for catheterization included the characterization of LV outflow gradient, the assessment of children considered to be at high risk for sudden death, and for symptoms such as chest pain, dyspnea and syncope. The 22 children assessed after this time were a consecutive series and had similar clinical features. Henceforth, no distinction is made between these groups. There was a high prevalence of risk factors associated with sudden death (Table 1).

Prevalence, severity, and distribution of bridging. Myocardial bridging is illustrated in Figure 1. A total of 28 bridges were present in 23 (40%) of the children. Multiple bridging sites were identified in 4 (7%) of the 57 children: 2 coronary segments in 3 patients, and 3 segments in 1 patient. Bridging was located at mid-LAD in 13 (46%), proximal LAD in 1 (4%), distal LAD in 2 (7%), a diagonal branch in 5 (18%), an obtuse marginal branch in 4 (14%), and the posterior descending branch of the right coronary artery in 3 (11%) of all 28 bridges. The mean length of the compressed coronary segment was 15 ± 7 mm, range 6 to 39 mm, with a mean systolic narrowing of 76 ± 18%. The severity of maximum compression was between 50% and 75% in 15 (54%) and 75% and 100% in 13 (46%) of the bridges. Complete systolic occlusion of the coronary lumen was seen in 8 (29%) of the bridges.

The relation of bridging to clinical findings. The clinical correlates of bridging are shown in Tables 2 and 3. The ages at diagnosis of HCM and cardiac catheterization were similar in the children with and without bridging. Clinical presentation (chest pain and symptoms of impaired consciousness, including cardiac arrest) was similar in the two groups.

ECHOCARDIOGRAPHY. LV wall thickness at proximal interventricular septum was significantly greater in the children with bridging; the mean septal thickness in children

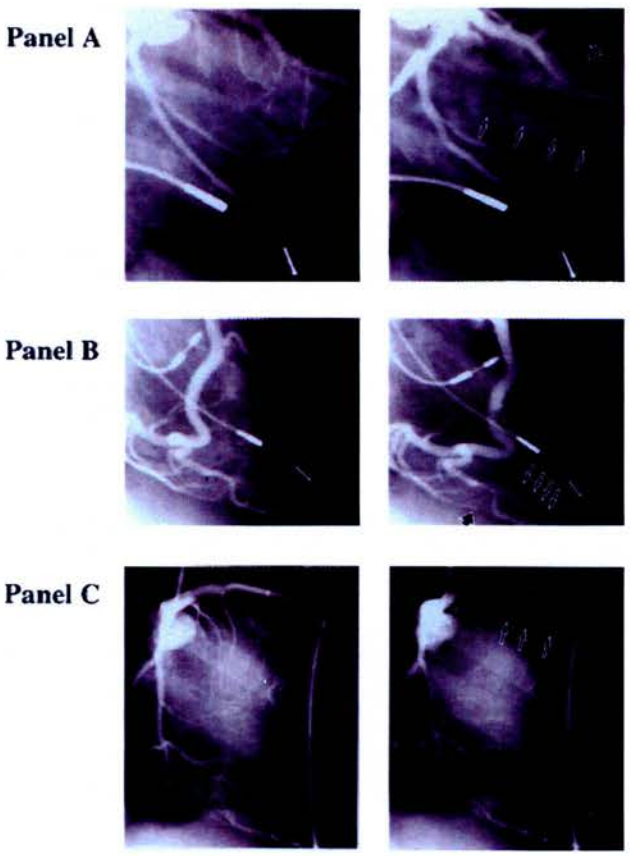

Figure 1. Figure illustrating coronary angiograms in three children with hypertrophic cardiomyopathy. Diastolic frames are on the left and systolic frames on the right. **Panel A** = 6-year-old male with mid-left anterior descending artery (LAD) bridging and compression of its intramyocardial branches. **Panel B** = 13-year-old female with bridging of proximal posterior descending branch of the right coronary artery with complete compression of its septal branches. **Panel C** = Complete compression of septal branches of the LAD. This child had bridging of an obtuse marginal branch. Large arrows indicate sites of bridging of epicardial arteries and small arrows indicate compression of septal branches.

with bridging was 28 ± 8 mm compared with 19 ± 8 mm in children without bridging, $p < 0.001$. Furthermore, children with bridging had a greater degree of asymmetrical septal hypertrophy and higher LV outflow gradients (Table 2, Fig. 2). The diastolic LV cavity in children with bridging was 39 ± 7 mm in contrast to 43 ± 6 mm in children without bridging; $p < 0.05$.

HEMODYNAMIC VARIABLES. At cardiac catheterization, 15 (65%) of the 23 children with bridging had LV outflow obstruction at rest or following provocation compared with 13 (38%) of the 34 children without bridging, $p = 0.08$. Mean resting LV outflow gradient was greater in patients with bridging than without (37 ± 34 mm Hg vs. 14 ± 24 mm Hg, $p = 0.005$). The association between LV outflow obstruction and bridging was further demonstrated by significantly higher LV systolic pressure in affected children (Table 2, Fig. 2).

EXERCISE THALLIUM SCINTIGRAPHY. Reversible myocardial abnormalities were present in 31 (65%) of 48 children

Table 2. Clinical Findings in HCM Children With or Without Myocardial Bridging (≥50% Max. Systolic Compression)

	All Children n = 57	Absent n = 34	Present n = 23	p Value
Age (yrs)				
At diagnosis (range)	10 ± 6	11 ± 6 (0.03–18)	10 ± 6 (0.1–17)	0.23
At angiography (range)	15 ± 4	15 ± 4 (6–20)	14 ± 4 (4–20)	0.35
Follow-up (yrs)				
From diagnosis	8 ± 6	9 ± 6	8 ± 6	0.7
From catheterization	4 ± 4	5 ± 4	3 ± 3	0.08
Gender				
Males	43 (75%)	28 (82%)	15 (65%)	0.25
Family history of HCM	25 (44%)	13 (38%)	12 (52%)	0.44
Family history of sudden death	12 (21%)	8 (24%)	4 (17%)	0.74
Clinical presentation				
Asymptomatic	10 (18%)	6 (18%)	4 (17%)	0.99
Chest pain	33 (58%)	20 (59%)	13 (57%)	0.92
Dyspnea	31 (54%)	18 (53%)	13 (57%)	0.99
Presyncope	29 (51%)	16 (47%)	13 (57%)	0.67
Syncope	23 (40%)	15 (44%)	8 (35%)	0.67
Cardiac arrest	4 (7%)	3 (9%)	1 (4%)	0.64
Echocardiographic indices				
Aorta (mm)	29 ± 4	29 ± 4	29 ± 4	0.87
Left atrium (mm)	39 ± 7	38 ± 7	39 ± 8	0.63
LVIDd (mm)	42 ± 7	43 ± 7	39 ± 6	0.02
LVIDs (mm)	23 ± 6	24 ± 6	21 ± 6	0.06
Interventricular septum (mm)	23 ± 9	19 ± 8	28 ± 8	0.0004
Posterior LV wall (mm)	11 ± 3	12 ± 4	11 ± 3	0.43
Septum to posterior LV wall ratio	2.2 ± 1.1	1.8 ± 0.9	2.7 ± 1.2	0.001
LV outflow gradient (mm Hg)	28 ± 35	16 ± 28	45 ± 37	0.002
Cardiac catheterization				
Right atrium (mm Hg)	6 ± 3	6 ± 3	5 ± 3	0.88
Systolic right ventricle (mm Hg)	34 ± 10	33 ± 9	36 ± 11	0.23
Mean pulmonary artery (mm Hg)	18 ± 7	18 ± 7	18 ± 6	0.99
Cardiac index (l/min/m^2)	3.1 ± 0.7	3.0 ± 0.6	3.2 ± 0.8	0.30
PCWP (mm Hg)	12 ± 5	12 ± 5	12 ± 5	0.67
Systolic LV (mm Hg)	123 ± 28	116 ± 21	133 ± 34	0.03
LV end-diastolic pressure (mm Hg)	16 ± 8	16 ± 9	17 ± 8	0.77
Mean aortic pressure (mm Hg)	72 ± 12	75 ± 13	68 ± 10	0.04
LV outflow gradient (mm Hg)	23 ± 31	14 ± 24	37 ± 34	0.005
Number with septal compression (%)	37 (65%)	14 (41%)	23 (100%)	< 0.0001

LVIDd = diastolic LV internal dimension; LVIDs = systolic LV internal dimension; PCWP = pulmonary arterial capillary wedge pressure.

who underwent thallium studies (Table 3, Fig. 2). Abnormalities in myocardial thallium uptake were also significantly associated with increased LV wall thickness (septum, 26 ± 9 mm vs. 16 ± 3 mm, p = 0.0001; higher ratio of septum:posterior LV wall thickness, 2.4 ± 1.1 mm vs. 1.4 ± 0.5 mm, p < 0.005), and greater LV outflow gradients at cardiac catheterization (26 ± 33 mm Hg vs. 7 ± 12 mm Hg, p < 0.05). Multivariate analysis demonstrated that LV septal thickness and septal perforator compression, and not LV obstruction or bridging, were independent predictors of reversible myocardial thallium uptake abnormalities (Table 4). After adjustment for confounding factors, the study has a power of 90% at 5% significance level to detect an odds ratio of 3 for a bridging effect on thallium abnormalities.

Thallium myocardial abnormalities most frequently affected the septum and anterior segments, the LV wall segments supplied by the most frequently bridged vessel. However, segmental defects in thallium uptake are often unrelated to the coronary artery compressed. The pattern of abnormalities on thallium scintigraphy is no different between the 16 children with LAD compression and the 7 with bridging of another coronary. Furthermore, stress-induced apparent LV cavity dilation, suggesting subendocardial ischemia, is a finding that cannot easily be attributed to bridging and was present in more than a third of the children. Thus, the distribution of myocardial thallium defects indicates the importance of factors other than bridging in etiology of the myocardial ischemia.

EXERCISE TESTS. There were no significant differences in mean exercise durations, resting and peak systolic blood pressure in children with and without bridging (Table 3).

ELECTROCARDIOGRAPHIC VARIABLES. The mean QT and QTc intervals and QTc dispersion were not significantly different in children with and without bridging (Table 3).

Table 3. Clinical Findings in HCM Children With or Without Myocardial Bridging (≥50% Max. Systolic Compression)

	All Children	Intramyocardial Bridging		p Value
		Absent	Present	
Treadmill exercise test	(n = 52)	(n = 32)	(n = 20)	
Exercise duration (s)	511 ± 228	534 ± 254	474 ± 178	0.36
Baseline heart rate (beats/min)	83 ± 14	82 ± 15	84 ± 12	0.48
Peak heart rate (beats/min)	167 ± 35	167 ± 39	166 ± 28	0.93
Baseline systolic BP (mm Hg)	123 ± 20	129 ± 19	114 ± 19	0.007
Peak systolic BP (mm Hg)	149 ± 43	154 ± 45	142 ± 38	0.32
Abnormal exercise BP response	26 (50%)	16 (50%)	10 (50%)	0.77
Exercise thallium scintigraphy	(n = 48)	(n = 30)	(n = 22)	
Abnormal study	31 (65%)	14 (47%)	17 (94%)	0.002
Number of abnormal segments	86/288 (30%)	34/180 (19%)	52/108 (48%)	< 0.0001
Perfusion abnormality				
Anterior	25	11	14	0.01
Septum	21	6	15	0.003
Subendocardium*	18	7	11	0.01
Inferior	11	4	7	0.17
Apex	9	4	5	0.47
Lateral	2	2	0	0.50
12-lead ECG	(n = 53)	(n = 32)	(n = 21)	
QT interval (ms)	433 ± 59	438 ± 52	425 ± 67	0.26
QTc interval (ms)	475 ± 50	471 ± 56	482 ± 40	0.31
QTc dispersion (ms)	40 ± 27	38 ± 25	42 ± 30	0.59
Ventricular tachycardia				
On Holter	7/42 (17%)	3/23 (13%)	4/19 (21%)	0.68
Induced at EP study	5/36 (14%)	3/24 (13%)	2/12 (17%)	0.99

*Reversible apparent LV cavity dilation.
BP = sphygmomanometer cuff blood pressure; EP study = electrophysiologic study.

AMBULATORY HOLTER MONITORING AND ELECTROPHYSIOLOGIC STUDIES. Nonsustained VT was recorded in 17% of the children during ambulatory ECG monitoring. Sustained VT was also induced in 17% of the children. Differences in VT on Holter and VT induced at EP study in children with or without bridging were not statistically significant (Table 3).

Clinical outcomes. Cardiac events (sudden death [n = 2], cardiac arrest [n = 4]) occurred in two children with bridging and in four without. The cumulative survival rate at 20 years of age in both children with bridging was 85 ± 10% and without bridging 82 ± 8%, p = 0.9 (Fig. 3). In addition, LV systolic dysfunction occurred in three children without bridging and in one child following transmural myocardial infarction (none in children with bridging). One child in each group underwent cardiac transplantation for severe symptoms and exercise limitation.

Compression of septal branches of the LAD. Children with partial or complete compression of septal branches of the LAD had significantly greater LV wall thickness, asymmetrical septal hypertrophy, thallium perfusion defects and LV obstruction compared with children without compression (Table 5). Septal compression occurred in all of the 23 children with bridging but in only 14 of the 34 children without bridging, p < 0.001. QT intervals, VT on Holter or induced at EP study, and prognosis were not significantly different in children with or without compression of the septal branches.

DISCUSSION

Several mechanisms have been postulated to explain an increased incidence of sudden death in HCM. These include ventricular arrhythmias, an atrial arrhythmia causing severe hypotension because of associated LV dysfunction, bradyarrhythmias, LV outflow obstruction and myocardial ischemia (1,4).

MYOCARDIAL ISCHEMIA. More than half of patients with HCM have reversible myocardial perfusion abnormalities as demonstrated by exercise thallium or sestamibi studies, despite having large normal epicardial vessels with high flow velocity (1,3,4). The perfusion abnormalities are believed to represent regions of myocardial ischemia caused by one or more mechanisms (1–17).

PREVALENCE AND CLINICAL SIGNIFICANCE OF BRIDGING. Bridging is a common finding, seen in up to 10% of patients who undergo coronary angiography (21–24). It is observed in up to 40% of patients with chest pain and normal coronary arteries after administration of drugs such as nitroglycerin and isoproterenol (22). Muscle bridges are identified at autopsy or aortocoronary bypass surgery in 5% to 86% of cases (24–27). Bridging is more common in cardiac diseases that are associated with LV hypertrophy (23,27,28). Its angiographic prevalence in adult patients with HCM is about 30% to 80% (11,22). Compression of the intramyocardial septal arteries also occurs more fre-

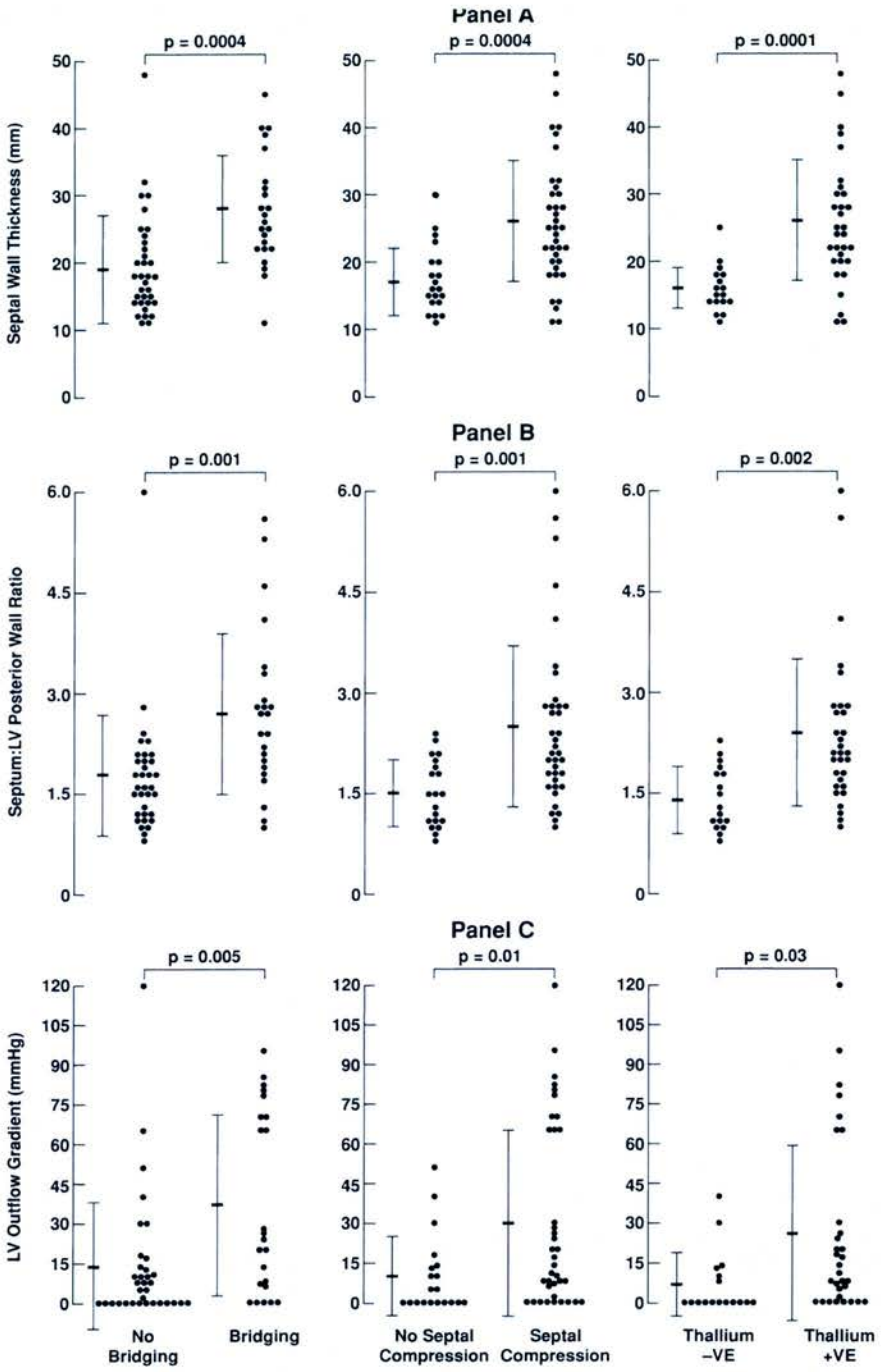

Figure 2. Figure showing interventricular septal wall thickness **(panel A)**, ratio of septum:posterior wall thickness **(panel B)**, and left ventricle outflow gradient at cardiac catheterization **(panel C)** in children with and without bridging of epicardial coronary arteries or compression of septal branches, and in children with and without myocardial perfusion abnormalities. Thallium (−ve), normal myocardial perfusion; thallium (+ve), abnormal myocardial perfusion.

quently in patients with LV hypertrophy. It is present in >70% of patients with aortic stenosis or HCM and may be related to severity of the LV hypertrophy (1,29).

Several reports have indicated that bridging may cause myocardial ischemia (13,24,25,28,30–32). Bridging has also been reported to cause myocardial infarction, abnormalities of the QT interval, VT and sudden death (13,33–37). Surgery to unroof or bypass the bridge and coronary artery stenting have also been described to improve symptoms in isolated cases and in small series of patients (13,38–41).

There is, however, as yet no convincing evidence that surgery improves morbidity or mortality in patients with bridging (24,27). Bridging may be an incidental finding in patients without another cardiac explanation for chest pain.

Table 4. Relation of Thallium Perfusion Abnormalities to Clinical Parameters: Logistic Regression Model for Predicting Thallium Perfusion Abnormalities

	Odds Ratio	p Value
Univariate analyses		
Interventricular septal wall thickness (per SD of 9 mm)	9.90	< 0.01
LV outflow obstruction (mm Hg)	1.02	< 0.01
Bridging (absent or present)	3.97	< 0.01
Septal perforator compression (absent or present)	7.30	< 0.01
Multivariate model with all 4 variables		
Interventricular septal wall thickness (per SD of 9 mm)	9.45	< 0.01
LV outflow obstruction (mm Hg)	1.01	0.2
Bridging (absent or present)	1.61	0.14
Septal perforator compression (absent or present)	3.05	< 0.05

SD = Standard deviation.

In several case reports myocardial hypertrophy was also present, and some of the patients in these reports may have had HCM. As only 15% of coronary flow occurs during systole, the physiologic relevance of bridging is questionable. Bridging may be postulated to cause myocardial ischemia if there is increased myocardial demand and/or diastolic compression of the coronary vessel. However, several studies have not demonstrated that bridging causes critical stenosis during diastole (17,22,30,31).

Yetman et al. (13) described a strong relation between bridging, chest pain, history of cardiac arrest, reduced exercise capacity, hypotensive responses to exercise, increased QTc dispersion, VT and poor prognosis in 36 children with HCM diagnosed and evaluated over about 40 years. They found no association between bridging and degree of LV hypertrophy or LV outflow obstruction. Following diagnosis with HCM, the five-year survival rate in children with bridging was 67% and 94% in the children without bridging. They concluded that bridging was an important cause of myocardial ischemia in their HCM children, and a direct determinant of clinical outcome. The

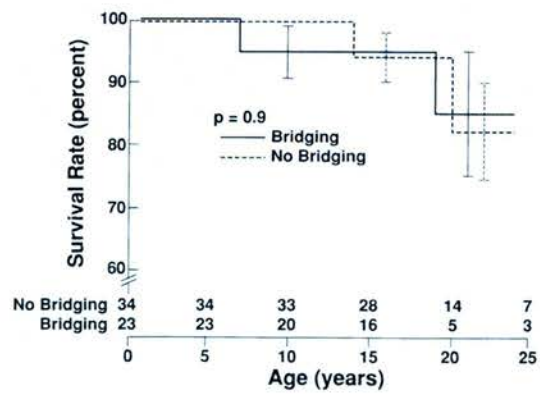

Figure 3. Figure showing survival rates in children with and without bridging. Cardiac death was defined as death or cardiac arrest. The number of children at different age intervals is indicated at the bottom of each panel.

Table 5. Clinical Findings in HCM Children With or Without Compression of Septal Branches of the LAD Artery

	Compression of Septal Branches		
	Absent	Present	p Value
Echocardiographic indices	(n = 20)	(n = 37)	
Interventricular septum (mm)	17 ± 5	26 ± 9	0.0004
Posterior LV wall (mm)	12 ± 4	11 ± 3	0.30
Septum:posterior wall thickness ratio	1.5 ± 0.5	2.5 ± 1.2	0.001
LV outflow gradient (mm Hg)	13 ± 29	35 ± 36	0.02
Cardiac catheterization	(n = 20)	(n = 37)	
Systolic aortic pressure (mm Hg)	108 ± 18	97 ± 12	0.01
Mean aortic pressure (mm Hg)	78 ± 13	69 ± 7	0.01
LV outflow gradient (mm Hg)	10 ± 15	30 ± 31	0.01
Coronary bridging	0/20	23/37 (76%)	< 0.0001
Exercise thallium scintigraphy	(n = 17)	(n = 31)	
Abnormal study	4 (24%)	27 (87%)	< 0.0001
Number of abnormal segments	9 (9%)	77 (41%)	< 0.0001

authors performed surgical unroofing of the myocardial bridge in three of the children and suggest that this procedure reduces myocardial ischemia. As thallium myocardial scintigraphy was available in less than a third of the children, the contribution of bridging to myocardial perfusion abnormalities was not adequately assessed.

The authors found coronary compression persisting through 50% of diastole, providing a credible mechanism for limitation of flow. Diastolic compression was calculated from the number of angiographic frames showing diastole and the proportion of these showing bridging (13). To allow this calculation, both the start and duration of diastole need to be accurately measured; this is difficult to do from angiographic records without simultaneous echocardiographic, electrocardiographic or phonocardiographic recordings. Coincidental quantitative coronary and diastolic information can be obtained from LV or aortic angiograms from the "nonselective" coronary runoff. This, however, may dramatically reduce sensitivity for the detection of moderate degrees of bridging. Using LV angiography may also result in detection sensitivities favoring detection of mid LAD compression, as this artery is seen more clearly than other epicardial arteries in right anterior oblique views and is seen at an orientation most likely to reveal eccentric compression.

Bridging is phasic, with transition from maximum to minimum compression. Identifying the end of the period of compression is therefore difficult and requires strict definition. Compression deforms the coronary lumen in an eccentric manner. The maximum compression is a measure of the short axis of an ellipse; lumen area is reduced according to elliptical and not circular geometry. For example, a concentric stenosis of 60% but an elliptical compression of 84% will reduce lumen area by 75% (27). Therefore,

regardless of the fraction of diastole during which compression persists, significant effects on flow will result only if compression remains severe.

Coronary compression increases with catecholamine administration (22). Therefore, coronary compression may be increased in frequency and severity during exercise when diastole is abbreviated. We assessed compression at rest and it is possible that during exercise the severity of compression may be flow limiting. Coronaries compressed at rest are likely to be those most severely affected during exercise. As the presence of bridging at rest does not predict myocardial perfusion abnormalities, it seems unlikely that the more frequently occurring bridging during exercise will do so. Furthermore, patterns of perfusion abnormality and artery affected were poorly matched.

Comparisons with similar studies. The present study was undertaken to investigate the prevalence of bridging and clinical significance of bridging and systolic compression of the septal branches of the LAD in children with HCM. We were unable to reproduce the findings of Yetman et al. (13). Bridging, despite identical definitions, was present more frequently, involved most epicardial arteries, and was occasionally at several coronary sites in the same child.

Exercise-induced reversible thallium myocardial perfusion abnormalities were more common in children with bridging and compression of septal branches. However, in contrast to the findings of Yetman et al. (13), bridging was associated with significantly greater LV hypertrophy, asymmetrical septal hypertrophy and LV outflow obstruction. Multiple regression analysis supported the hypothesis that the thallium abnormalities were related to severity of hypertrophy and presence of septal compression rather than caused by bridging. Symptoms, diminished exercise capacity, and abnormal blood pressure responses to exercise were not more common in children with bridging.

Yetman et al. (13) found that a QTc difference of >60 ms in any of the 12 ECG leads differentiated children with and without bridging with a sensitivity of 92% and a specificity of 77%. In our study, the QT, QTc, and dispersion of QTc intervals were not longer in the children with bridging. We were also unable to demonstrate a relation between bridging, spontaneous VT and VT induced at EP study, or cardiac events (sudden death, cardiac arrest, and myocardial infarction).

The differences between the findings of two studies may be explained in part by the fact that Yetman et al. (13) relied on echocardiographic and angiographic records extending over a period of 41 years from 1956 to 1997. Patient selection may have significantly influenced their findings; on average, in their group without bridging, HCM was diagnosed eight years earlier than in the group with bridging. The most rapid period of disease progression (LV hypertrophy and development of LV outflow obstruction) and highest incidence of sudden death is during the second decade of life. Calculation of survival rates from the time of diagnosis of HCM perpetuates the confounding effect of selection on the results. Indeed, symptoms, ventricular arrhythmias, cardiac arrest and sudden death are uncommon under 10 years of age. Hence, if the time interval from diagnosis of HCM is used to calculate survival, children aged 3.3 (median) years would be expected to have a better prognosis than those diagnosed at 11.2 years, irrespective of the presence or absence of factors such as bridging. In our study, children with and children without bridging were diagnosed and evaluated at similar ages, and we calculated age-related outcomes.

An important consequence of the long duration of the Yetman et al. (13) study is that the management of some of the children in that series predated the advent of beta-blocker and verapamil therapies, which are now used routinely to manage symptoms and myocardial ischemia in children with HCM. These children were severely affected with a high prevalence of risk factors for sudden death (Table 1). The better survival rates in our series may reflect more current methods of management.

Conclusions. Bridging was present in about half of our children with HCM. Mid-LAD was the most commonly affected vessel but other coronary arteries were also involved. Compression of septal branches of the LAD was present in 65% of the children. Both bridging of epicardial arteries and systolic compression of the septal branches were related to LV hypertrophy and LV outflow obstruction. Thallium myocardial perfusion abnormalities were related to LV hypertrophy, LV outflow obstruction, bridging and systolic compression of the septal perforators. Multivariate analysis identifies only LV hypertrophy and septal compression as independent predictors of thallium abnormalities. No significant association was found between bridging and symptoms, ventricular arrhythmias and incidence of sudden death. These findings suggest that cardiac surgery (or stenting) for bridged arteries in children with HCM is not indicated. Verapamil and beta-adrenergic receptor blockade therapy and relief of LV outflow obstruction may provide adequate anti-ischemic therapy.

Study limitations. The present study consists of a selected subset of children with HCM who were evaluated by cardiac catheterization and, in many cases, by EP studies in part because of severity of their clinical presentation. Our standard practice has been to assess the coronary circulation in all children undergoing catheterization to determine the gross coronary anatomy; in many children LV or aortic root angiography was considered to sufficiently visualize the proximal coronaries. A smaller proportion required selective coronary angiography for adequate characterization. Only this latter group has been considered for the current study, as nonselective studies do not have the coronary resolution required for evaluating the distal coronaries and for quantitative analysis. Nonetheless, the children studied are a subset of a population referred for tertiary evaluation and thus are not considered representative of the larger population of HCM children. It is likely, however, that our population of children more accurately reflects current

patterns of diagnosis and referral than that in the study of Yetman et al. (13). The prevalence of bridging in an unselected population of HCM children is expected to be lower in keeping with less severe cardiac hypertrophy. However, it is likely that the relation of bridging to severity of LV hypertrophy and myocardial perfusion abnormalities, and the finding that bridging does not significantly determine clinical outcome, may also apply to HCM children who attend nontertiary cardiac centers.

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